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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **315th Meeting** *of the Biochemical Society was held in the London School of Hygiene and Tropical Medicine, Keppel Street, London, W.C.* 1, *on Saturday,* 21 *February* 1953, *at* 11.0 *a.m. and took the form of a Symposium on:*

"BIOLOGICAL TRANSFORMATIONS OF STARCH AND CELLULOSE"

COMMUNICATIONS

The Biological Synthesis of Starch. BY E. J. BOURNE. (*Chemistry Department, University of Birmingham*)

A review will be given of the methods by which unbranched 1:4-α-glucosans of the amylose class have been synthesized *in vitro*. These include the following syntheses: (*a*) by phosphorylase from dipotassium glucose-1-phosphate, (*b*) by amylomaltase from maltose, (*c*) by Schardinger dextrinogenase from *cyclo*amyloses, and (*d*) by amylosucrase from sucrose. The formation of amylopectin and glycogen from amylose, by means of *Q*-enzyme and Cori's 'branching factor', will be discussed. It will be shown that all of these conversions involve transglycosylation, and thus fall into line with polysaccharide syntheses in general. Such reactions entail retention of the initial ring size and of the anomeric linkage. Attention will be drawn to the specificity of the enzymes and to the reversibility of the reactions. Some new results with the *Q*-enzyme of *Polytomella coeca* will be presented.

The Enzymic Breakdown of Starch. By W. J. WHELAN. (*Department of Chemistry, University College of North Wales, Bangor*)

The enzymes to be discussed under this heading are those which initiate reaction between starch molecules and water, resulting in the scission of glycosidic linkages and an increase in the number of free reducing groups. These comprise enzymes hydrolysing (i) α-1:4-linkages, (ii) α-1:6-linkages, (iii) α-1:4 and 1:6-linkages, and (iv) the β-linkages present in amylose. In their actions the enzymes fall into two groups, (*a*) enzymes able to penetrate the molecule and, subject to certain limitations, hydrolyse any appropriate linkage, e.g. α-amylase, *R*-enzyme, and (*b*) enzymes which can only hydrolyse linkages exposed at terminal non-reducing chain ends, e.g. β-amylase, amylo-1:6-glucosidase, glucamylase.

Recent unpublished work defining the action patterns and specificities of α-amylase, β-amylase and *R*-enzyme will be described, together with an account of the use of these enzymes in exploring the fine structures of ramified starch-type polysaccharides and in determining the proportions of branch linkages. A new type of enzyme action will be reported in which the presence of two active enzyme preparations (α-amylase and *R*-enzyme) is necessary before hydrolysis of the substrate takes place.

The possible *in vivo* functions of some of these enzymes will be discussed.

Starch Synthesis and Degradation *in vivo*. By HELEN PORTER. (*Research Institute of Plant Physiology, Imperial College of Science and Technology, London, S.W.* 7)

Starch synthesis occurs in plants when supply of primary assimilate exceeds capacity for utilization in growth; thus under conditions of nitrogen deficiency starch levels are high.

Starch formation may be induced in the dark, from sugars and related compounds, in most leaves, whether or not they normally contain starch. Etiolation does not prevent synthesis, and oxygen is necessary for both synthesis and degradation (Nurmia, 1935).

α-Amylase and phosphorylase are widely distributed, but these enzymes do not appear to attack the native starch grain, nor do the products of their action upon starch *in vitro* accumulate *in vivo*. Sucrose is the principal intermediate formed *in vivo*, and recently production of phosphate esters of glucose and fructose has been reported. The mode of synthesis of sucrose and its quantitative relation to starch formation is an outstanding problem of carbohydrate metabolism (Hassid & Putman, 1950;

Albaum, 1952). The presence of a thermolabile inhibitor, probably α-amylase, prevents synthesis of polysaccharide from glucose-1-phosphate (G-1-P) by many leaf extracts; similarly, β-amylase inhibits phosphorylase, and such interactions may be one way in which synthesis is regulated (Porter, 1952). Leaf disks will effect polysaccharide synthesis from G-1-P at the cut edges, and throughout the disk if entry is facilitated by pretreatment with toluene. The product is probably amylose (Stumpf, 1952; van Fleet, 1952). Toluene treatment, or addition of phosphate, at pH 7·0, inhibit synthesis in leaf disks, from simple sugars. Concentration of phosphate determines the degree of inhibition. Preliminary experiments using ^{14}C-labelled sugars indicate that fructose and glucose supplied together are equally available for starch synthesis, and that sucrose concurrently synthesized is labelled equally in both moieties, whichever hexose is initially labelled.

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The Chemistry and Chemical Degradation of Cellulose. By G. O. Aspinall. (*Chemistry Department, King's Buildings, Edinburgh*)

Cellulose, the chief constituent of the cell walls of the majority of plants, consists of long chains of 1:4-linked β-D-glucopyranose units. That over 99% of the linkages are of this type is indicated by the high yield of 2:3:6-trimethyl D-glucose on hydrolysis of methylated cellulose and confirmed by studies of the kinetics of this reaction. Chemical measurements of chain length by the end-group method indicate a minimum value of 1700 glucose residues, and physical measurements of molecular size give values of 3000 or greater for the degree of polymerization of native cellulose.

X-ray studies show that the length of the unit cell along the axis of the cellulose fibre corresponds to that of two glucose residues linked as in cellobiose and X-ray studies together with chemical studies show that the fibre contains crystalline and amorphous regions. Hydrogen bonding occurs between the hydroxyl group of adjacent cellulose chains in the crystalline region, and the high tensile strength of cellulose fibres can be explained on the assumption that cellulose chains run through two or more crystalline regions.

Chemical degradation of cellulose occurs with acids and with oxidizing agents. The mechanical strength of the fibres disappears on treatment of cellulose with dilute acids with the formation of hydrocellulose (D.P. *c.* 200). The products of oxidative breakdown are either acidic in character due to oxidation of the $—CH_2OH$ groupings or aldehydic in the case of glycol-splitting reagents. Preferential reaction of certain residues in the cellulose chain has been explained chemically on the assumption of periodic modified linkages and physically on the basis of the greater accessibility of the amorphous regions.

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Cellulases. By M. V. Tracey. (*Rothamsted Experimental Station, Harpenden, Herts*)

Until about 20 years ago the snail was the source of material for work on cellulase. In the recent war textile deterioration in the tropics assumed great economic importance; consequently knowledge of micro-organisms producing powerful cellulases was rapidly expanded. Recently, research on rumen digestion has added further to our knowledge of microbiological cellulase producers. It is only in these two fields that our knowledge of the distribution of cellulase is at all thorough. The known distribution of cellulase among other organisms is extremely patchy. Differentiation between cellulase

production by an organism with a gut and production of cellulase by organisms living in that gut is often difficult in the smaller invertebrates. It seems likely, however, that cellulases are produced by some molluscs and arthropods.

The first report of the preparation of a cellulase in an apparently pure state appeared a year ago (Whitaker, 1951). For the first time some evidence can be produced about the course of enzymic cellulose breakdown. It had been, and still is, assumed that this is formally similar to the breakdown of amylose by β-amylase and maltase. More recent work has led to the supposition that an additional 'disaggregating' enzyme is involved. Whitaker's results using purified *Myrothecium* cellulase suggest that this enzyme is capable alone of the complete hydrolysis of cellulose to glucose and that interpretation of results of the action of crude extracts may be made uncertain by the absorption phenomena inevitable in the action of an enzyme on an insoluble polymeric substrate.

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Digestion of Cellulose by Ruminants. By A. T. Phillipson. (*Rowett Research Institute, Bucksburn, Aberdeenshire*)

The ability of the ruminant to digest large quantities of cellulose is the result of the activity of the bacterial population that inhabits the alimentary tract and, in particular, the rumen. Crude fibre as analysed by the current agricultural procedures is a fair measure of cellulose, and the two terms will be considered as synonymous. In spite of the importance of cellulose as a foodstuff to ruminants, very little is known of the stages of its digestion in the rumen. The end-products of fermentation found by the *in vitro* incubation of filter-paper by rumen bacteria appear to be largely acetic and propionic acids and carbon dioxide (Elsden, 1946; Marston, 1948); the intermediate stages that occur in the rumen are unknown, except that succinic acid is known to be a precursor of at least some of the propionic acid (Sijpesteijn & Elsden, 1952). It is noteworthy that acetylated or methylated celluloses are indigestible.

When natural fodders are fed potentially digestible fibre is excreted in the faeces, and although some 45–60 % of the total fibre disappears as it passes through the alimentary tract more could do so if it remained in the rumen long enough. Further digestion occurs in the caecum and colon, but this is slight compared to digestion in the rumen and does not remove all the potentially digestible fibre. Finely divided particles of food pass through the rumen more rapidly than large particles when both are fed together, but if the whole ration is ground it remains there for an abnormally long period and, strangely enough, fibre is less completely digested (Balch, 1950). The reason for this is not known.

Digestion of fibre is lowered by several other factors. Feeding starch or other soluble carbohydrates in quantity depresses the digestion of fibre, although this can to some extent be counteracted by increasing the protein of the feed. Increasing protein alone, however, also depresses fibre digestion, even though the fodder by itself is low in nitrogen. Cellulose-fermenting bacteria apparently receive adequate nitrogen from the fodder itself. Only one dietary factor increases digestion of fibre: adding the ash of lucerne at the rate of 3·7 % to a ration containing coarse fodder and a total fibre content of 17·8 % increases digestion of fibre by some 10 %; methane excretion is also increased (Swift, Cowan, Barron, Maddy & Grose, 1951). It is not known which of the mineral constituents of this ash is responsible for this effect. Rumen fluid normally contains nearly as much potassium as sodium, and only small concentrations of soluble calcium and magnesium. In contrast lucerne ash is rich in calcium and magnesium; sufficient data is not available to make a direct comparison of all inorganic ions and little is known of the trace element composition.

It seems probable from these facts that cellulose-fermenting bacteria are influenced by the inorganic composition of the media in which they live and, consequently, laboratory media for the cultivation of cellulose-fermenting bacteria should conform to the conditions in the rumen; this has been generally recognized as far as phosphorus is concerned but not as regards cations. The results of adding lucerne ash to the feed suggest that the only practical way of increasing cellulose fermentation in the rumen—apart from chemical treatment of the natural fodder—appears to be to produce optimal conditions by the judicious use of mineral supplements once the optimal conditions are known.

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The Occurrence and Prevention of the Biological Attack of Cellulose Textile Fibres. By R. G. FARGHER. (*Shirley Institute, Didsbury, Manchester* 20)

Since 1939, the pioneer work on the occurrence and prevention of the biological attack of cellulosic fibres, most of it undertaken in this country, has been extended considerably, particularly in the United States. Nevertheless, the biological techniques have been so variable and the failure adequately to characterize the substrates so frequent that the available data permit only a qualitative interpretation.

To the textile technologist, biological attack has two connotations. The first, and most common, is disfigurement without appreciable change in strength. The second, loss of strength ('tendering') of the fibres, is encountered less frequently, principally because of the high moisture requirement of the organisms which attack cellulose.

Apart from temperature and moisture content, the attack of cellulosic fibres by micro-organisms is controlled by the purity and crystallinity of the cellulose. It is very localized and occurs most readily in the least crystalline portions. As these are the most chemically reactive, resistance to attack can be enhanced by partial esterification—or by association with certain hydrous metallic oxides.

Resistance may also be increased by the 'barrier' protection claimed for the lignin component of the bast fibres or by the incorporation or organic antiseptics. The efficacy of insoluble copper compounds in 'burial' tests may afford a good example of barrier protection. The organic antiseptics present two interesting problems, namely, the very limited relationships between chemical constitution and toxicity and the comparative ease with which some of them are decomposed by micro-organisms.

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PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **Annual General Meeting** *of the Biochemical Society was held in the Department of Biochemistry, University College, Gower Street, London, W.C.* 1, *on Friday,* 27 *March* 1953, *at* 11.0 *a.m., when the following papers were read:*

COMMUNICATIONS

Spirilloxanthin Synthesis by Resting Cells of *Rhodospirillum rubrum*. By T. W. Goodwin and H. G. Osman. (*Department of Biochemistry, The University of Liverpool*)

Rhodospirillum rubrum synthesizes considerable amounts of spirilloxanthin when cultured anaerobically in the light on a malate/glutamate/salts/biotin medium (Goodwin & Osman, 1952, 1953). Cells cultured in this way and transferred when 3–4 days old to a medium containing an assimilable carbon source (e.g. acetate, malate, succinate), but no nitrogen, do not synthesize spirilloxanthin; if they are transferred to a similar medium containing a nitrogen source, then, as the cells multiply, pigment synthesis is resumed.

When *R. rubrum* is grown on the malate/glutamate medium containing diphenylamine (DPA) (1/140 000), then almost no spirilloxanthin (or bacteriochlorophyll) is produced, although growth is only slightly inhibited. Four-day-old cells from such cultures, washed free from DPA, resuspended in phosphate buffer and incubated anaerobically in the light immediately begin to synthesize spirilloxanthin (and bacteriochlorophyll), and within 24–48 hr. its concentration approaches that of normal anaerobic-light cultures. Similarly, washed DPA cultures in phosphate buffer synthesize the same amount of pigment when incubated aerobically in the dark, although normal aerobic-dark cultures produce much less pigment than do anaerobic-light cultures. Furthermore, little pigment is produced by DPA cells cultured anaerobically in the dark and an intermediate amount when cultured aerobically in the light.

It appears that DPA cultures accumulate a colourless material which can be converted into spirilloxanthin only when energy is available. This can be provided photo-chemically (anaerobically in the light) or by respiration (aerobically in the dark). This would explain the failure to form pigment anaerobically in the dark. The reduced production in aerobic-light cultures, is probably due to destruction of the pigment in the presence of both light and oxygen. In this connexion it has been found that resting DPA cultures take up oxygen rapidly whilst normal resting cells do not.

No carbon source yet examined (malate, acetate, succinate) will stimulate spirilloxanthin synthesis in washed DPA cells above that obtained on buffer alone. A possible explanation for this will be discussed.

Preliminary observations suggest that the accumulated precursor is probably not a saturated spirilloxanthin, although phytofluene (dodecahydrolycopene), absent from normal cultures, has been detected in DPA cultures in minute traces. As both spirilloxanthin and bacteriochlorophyll synthesis are equally inhibited by DPA, the possibility exists that DPA exerts its major effect either far back in the synthetic chains (e.g. in the utilization of a common precursor such as acetate), or on a mechanism common to both chains but involving different substrates.

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The Adrenergic Amines in Human Blood. By H. Weil-Malherbe. (*Research Department, Runwel Hospital, near Wickford, Essex*)

The concentration of adrenergic amines in plasma. Further evidence was sought for the identity with adrenaline and noradrenaline of the plasma components estimated in the method of Weil-Malherbe & Bone (1952). Plasma was extracted with *n*-butanol and re-extracted into 0·05 N-HCl. After adjusting the pH to ~3 with Dowex 2, the extracts were concentrated and purified by adsorption on, and elution from, a column of alumina. The recovery of chromogenic material at this stage was 80–90 %. Paper chromatography showed only two zones of chromogenic material with the R_F values of

adrenaline and noradrenaline. Both components were estimated separately after elution from the appropriate paper sections. In this way a mean adrenaline concentration of 2 μg. and noradrenaline concentration of 7 μg. per l. was found for plasma.

The concentration of adrenergic amines in erythrocytes. Difficulties previously experienced (Weil-Malherbe & Bone, 1952) were due to incomplete haemolysis. After haemolysis of washed erythrocytes with cetavlon or butanol, analyses were carried out successfully both by the original method and after paper chromatography of *n*-butanol extracts. The concentrations of adrenaline and noradrenaline were found to be about equal to those in plasma. The amines seem to be diffusible from erythrocytes, since dialysis of the cells against isotonic saline results in a marked lowering of their concentration.

Our experiments, which included partial hydrolysis of plasma with acid at 100°, have so far shown no indication for the presence in blood of a fraction with the properties of 'adrenalinogen' (Lehmann & Kinzius, 1951) or of 'protein-bound adrenaline' (Annersten, Grönwall & Köiw, 1949).

The simultaneous estimation of adrenaline and noradrenaline in one sample. A method is at present under investigation which we hope will make this possible. It is based on differences in the fluorescence spectrum of the derivatives of adrenaline and noradrenaline.

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The Amination of Inosinic Acid in Brain. By H. Weil-Malherbe. (*Research Department, Runwell Hospital, near Wickford, Essex*)

In a rat-brain homogenate prepared in distilled water, an initial concentration of 20–25 mg. NH_3/100 g. fresh tissue is found, and this value does not rise significantly on subsequent incubation. If isotonic saline is used for homogenization the initial NH_3 concentration is about 15 mg. %, and it increases to about 40 mg. % after 5 hr. incubation. In rat-brain slices, lastly, the NH_3 concentration rises from an initial value of 10 mg. % to about 56 mg. % after a 5 hr. incubation without substrate. These results may be interpreted as indicating that a part of the NH_3 formation accounting for about 20–30 mg. % is autolytic and another part is dependent on the integrity of structural units. Evidence has been obtained suggesting that the reaction depending on structure is an oxidative phosphorylation.

In rat slices incubated for 5 hr. without substrate there is a considerable increase of N.P.N. arising mainly from proteolytic reactions. Though products of proteolysis are presumably the ultimate sources of the NH_3 produced, they cannot be its immediate precursors as the requisite deaminases do not occur in brain. Of the five NH_3-producing enzymes occurring in brain, viz. glutamic dehydrogenase, glutaminase, amine oxidase, adenylic deaminase and adenosine deaminase, all but the last two could be eliminated with a fair degree of probability as immediately responsible for the non-autolytic NH_3 formation of brain slices.

It has now been found that addition of glutamine and either inosine triphosphate (ITP) or a mixture of inosinic acid and creatine phosphate to washed or dialysed brain homogenates, leads to an NH_3 formation considerably exceeding that of all possible control experiments. This result is interpreted as indicating the transfer of the amide group of glutamine to ITP or to another inosine phosphate, possibly at the expense of high-energy phosphate bonds, a reaction foreshadowed by the experiments of Kalckar & Rittenberg (1947).

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Transport of Water, Sodium, Potassium and α-Ketoglutarate in Kidney Cortex Slices. By R. WHITTAM and R. E. DAVIES. (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, University of Sheffield*)

Kidney slices can use metabolic energy to pump out water and Na, and accumulate K (Robinson, 1950; Mudge, 1951; Krebs, Eggleston & Terner, 1951), and these investigations have now been extended by simultaneous measurements of the movements of Na, K, water and substrate.

Guinea-pig kidney cortex slices were incubated in a physiological saline containing 155 mM-Na, 6 mM-K and 10 mM-α-ketoglutarate. The *in vivo* water content of the tissue was 77·7 %; the Na, K and α-ketoglutarate concentrations were 81·5, 98·1 and 0·15 mM (mmoles/kg. tissue water). After 3–6 min. on incubation in O_2 at 37° the Na increased to 125 mM and K decreased to 57 mM. After 20 min. these changes had been largely reversed to a steady state of 112 mM-Na and 96·0 mM-K, which was maintained for a further 30 min., representing an increase of 28·4 mM in the Na + K content. The water and α-ketoglutarate contents reached a steady state of 79·7 % and 14·8 mM. The gradient (concentration in tissue water/external concentration) was 1·30 for Na + K and 2·5 for α-ketoglutarate. Since no pH changes could be detected these results mean that for each molecule of (dibasic) α-ketoglutarate two sodium ions were accumulated. At pH values above 8 and below 6 the water content of the tissue progressively increased and the gradients of Na, K and Na + K changed towards unity.

Slices incubated 35–50 min. in O_2 or N_2 at 0° and in N_2 at 37° lost K and gained an approximately equivalent amount of Na, reaching a steady state of 34·6 mM-K and 135 mM-Na. The Na + K and α-ketoglutarate gradients became 1·05 and 0·33; the water content became 83·1 %. These slices, swollen at 0°, when incubated further in O_2 at 37° expelled water and Na, and accumulated K, whilst slices in a steady state at 37° lost K and gained Na and water when placed at 0°. In hypotonic medium (0·2 OS-M) at 0° the Na + K ratio was 1·26 (cf. Robinson, 1952).

The Na + K gradient at 0° in hypotonic saline represented a greater excess tissue Na + K (28 mM) than the excess (10 mM) in isotonic saline. This would not be expected if the gradient were due wholly to bound cations in the tissue. Thus, the prevention of swelling, and production and maintenance of Na and K gradients, are related labile properties which are dependent on temperature and pH and require a continuous supply of metabolic energy.

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Measurements of the Turnover-rates of Sodium and Potassium in Kidney Cortex Slices. By R. WHITTAM and R. E. DAVIES. (*Medical Research Council Unit for Research in Cell Metabolism, Department of Biochemistry, University of Sheffield*)

In previous experiments from this laboratory the turn-over rate of K in isolated tissue has been measured with ^{42}K (Krebs, Eggleston & Terner, 1951; Davies & Galston, 1951). The steady-state turn-over rates of Na and K in guinea-pig kidney cortex slices incubated in O_2 or N_2 at 0° and 37° have now been measured under the conditions given by Whittam & Davies (1953).

After 30–35 min. incubation either ^{42}KCl or $^{24}NaCl$ was added, the slices removed 2–6 min. later and turn-over rates (in μmoles/min./g. tissue) calculated from the formula of Krebs *et al.* (1951). The rates for K at 0° and 37° were 2·1 and 10·1 in O_2 and 1·5 and 2·7 in N_2. The rates for the exchange of K at 0° and 37° between Zeo-carb (10–30 mesh) and 10 mM-KCl were 192 and 297 μmoles/min./g. resin. 'Extracellular' Na, unlike K, is a major fraction of tissue Na, and the exchange between medium and 'extracellular' Na was fast with a half-life of less than 14 sec. The turn-over rates between 'intracellular' and 'extracellular' Na were calculated after correcting for this effect. At 0° and 37° in O_2 these turn-over rates were 6·8 and 13·5; in N_2 the rates were 5·5 and 7·9.

Thus, in kidney cortex slices, as in erythrocytes (Solomon, 1952), the Na flux is greater than the K flux. The effect of temperature on the Na and K exchanges in N_2 (44 and 80 % increase from 0° to 37°) was similar to its effect (55 %/37°) on K exchange in the resin. After allowance for changes

in tissue Na and K concentrations the rates at 37° in O_2 are higher (114 % for Na and 40 % for K) than might be expected if they were due to passive ion-exchange mechanisms (cf. Ussing, 1949). These results show that aerobic metabolism is needed for both the maintenance of concentration gradients and the rapid turn-over rates of Na and K in isolated slices, although a part is probably due to mechanisms not requiring energy.

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Biosynthesis of [^{14}C] β-Carotene. By J. Glover and E. R. Redfearn. (*Biochemistry Department, University of Liverpool*)

[^{14}C] β-Carotene is required for studies with experimental animals and also for the preparation of [^{14}C] retinene and [^{14}C] vitamin A. This communication describes preliminary work with detached tomato plant leaves. These have a relatively high β-carotene content (40–50 μg./g. fresh wt.). The immature top leaves with low carbohydrate reserves were taken from the plant about 8.00–8.30 a.m., divided into equal groups and floated on distilled water in glass culture-vessels. The latter were arranged in a closed system and the CO_2 removed by circulating the air with a small bellows pump through alkali absorbers. The leaves were then placed in the dark for 24 hr. to use up their remaining carbohydrate reserves.

Analysis of one group at this stage showed that the β-carotene concentration had fallen to 25–50 % of the original level in different runs. Then CO_2 (up to 0·3 % gas volume) was admitted to the remaining groups and circulated by the pump. The leaves were now illuminated by tungsten lamps (intensity 400 f.c.), and at the end of 24 hr. the β-carotene concentration had fallen a further 10 % of the starting level. With continued exposure to light, however, for another 48 hr., the β-carotene level returned to 80–90 % of its initial value.

That the increase in β-carotene in these later stages was due to synthesis *de novo* and not merely to regeneration from β-carotene epoxides was shown by comparing the concentrations of the latter with those of β-carotene at each stage. First, the increase in β-carotene-epoxide and -furanoid concentrations at the end of the dark period amounted to only a small fraction of the decrease in β-carotene. Secondly, illumination of the leaves brought the β-carotene-oxides back quickly to their normal level in the leaves, while the β-carotene concentration continued to fall. These results suggest that once β-carotene-epoxide or -furanoid is formed, it is not directly reduced to β-carotene again, but is oxidatively degraded further. New β-carotene is then synthesized from the smaller carbon compounds of the metabolic pool. This evidence that the synthesis of β-carotene follows a different route from that of its catabolism in the plant leaf is in agreement with the work of Bandurski (1949), who observed different temperature coefficients for β-carotene synthesis and degradation in bean leaves.

By depleting detached tomato plant leaves of their carbohydrate and β-carotene in the above manner, and allowing new β-carotene to be synthesized in the presence of $^{14}CO_2$, we have been able to obtain [^{14}C] β-carotene with a specific activity (μg./mg. C), approximately one-tenth that of the $^{14}CO_2$ in the vessel.

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Glycerylphosphorylethanolamine in Brain Tissue. By G. B. Ansell and Joyce M. Norman. (*Neuropsychiatric Research Centre, Whitchurch Hospital, Cardiff*)

The presence of free glycerylphosphorylethanolamine (GPE) in liver, previously demonstrated by Campbell & Work (1952), suggests that this substance may be an intermediate in the metabolism of phosphatidyl ethanolamine. An investigation of the metabolic activity of GPE in rat brain has therefore been carried out.

Two-dimensional paper chromatograms of rat-brain extracts prepared by mild procedures showed the presence of GPE in low concentration (2·3 mg.

%). This GPE is considered to be in the free state because experiments showed that the extraction methods employed did not release it from synthetic cephalin ($\alpha\alpha'$-dimyristyl cephalin) or a crude acetalphospholipin preparation, though prolonged treatment with 10% trichloroacetic acid solution at 4° would do so.

Furthermore, experiments in which $^{32}PO_4'''$ was injected intraperitoneally into young rats demonstrated that the specific activity of the GPE in brain extracts 1–4 hr. after injection was less than that of the ethanolamine *o*-phosphoric acid, but that after 24 hr. the two compounds were in isotopic equilibrium. This evidence also indicates that this GPE is a component of the acid-soluble phosphorus fraction. (Determinations of specific activity were carried out by methods similar to those used previously (Ansell & Dawson, 1951).) The specific activity of this GPE was always greater than that of the phosphatidyl ethanolamine determined concurrently by the method of Dawson & Norman (1953), from which it may be concluded that GPE is not a breakdown product of phosphatidyl ethanolamine. On the other hand, the specific activity of the GPE liberated by 10% trichloroacetic acid solution at 4° was always identical with that of phosphatidyl ethanolamine. This GPE may derive from acetalphospholipin, since this is known to be acid-labile (Feulgen & Bersin, 1939). However, the amount of acid-labile GPE obtained would indicate a concentration of acetalphospholipin in rat brain very much lower than the value usually accepted.

When minced rat brain was allowed to respire for short periods (1–2 hr.) in the presence of $^{32}PO_4'''$, there was a small but significant uptake of the isotope into the GPE present in the acid-soluble phosphorus fraction. This synthesis of radioactive GPE was increased by the presence of ethanolamine.

The results suggest that GPE may be an intermediary in the *in vivo* synthesis of phosphatidyl ethanolamine and the significance of the findings will be discussed.

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The Digestion of Intestinal Mucus. By H. G. Sammons. (*Department of Pharmacology, University of Birmingham*)

The fate of alimentary mucus is not fully understood, but it is not normally seen in any appreciable quantity in the faeces. Grossman & Hartiala (1952) state that trypsin but not pepsin will hydrolyse the protein moiety of mucus obtained from the duodenal pouch of a dog. In several specimens of duodenal juice obtained from human subjects we have been unable to obtain digestion of mucus by crystalline trypsin. Pancreatin in high concentration does act slowly on the mucus present in duodenal juice. Mucus obtained from an intestinal loop of a rat after oleic acid stimulation was not digested even by high concentrations of crystalline trypsin. Nevertheless, we have shown by the method previously described (Sammons, 1951) that trypsin will digest salivary mucus. It has now been shown that bile will inhibit this digestion, and it is thought that bile may play some part in the protection of intestinal mucus from digestion by trypsin.

There does, however, appear to be an enzyme present in the lower part of the small intestine which digests intestinal mucus. A proteolytic enzyme has been shown to be present in the discharge from ileostomies. This enzyme will digest all forms of mucus, and the presence of bile affords only slight protection when, as far as proteolytic activity is concerned, the activity as measured by the method of Tomarelli, Charney & Harding (1949), is the same as that of trypsin at a concentration of 10 mg./100 ml. water.

This mucoprotease is not normally present in the faeces. It was found, however, in some diarrhoeic stools and in the faeces of children. It has been found in the faeces of 3 out of 3 cases of confirmed pancreatitis when the duodenal enzymes have been completely absent.

This enzyme present in the ileostomy discharge is much more stable than trypsin. Vernon (1904) showed that the products of proteolysis will confer stability to trypsin, but the heat-inactivated extracts of the discharge do not confer stability to trypsin when added to its alkaline solution. Its origin appears to be confined to the lower end of the small intestine since it is not contained in gastric juice, duodenal juice or the rectal discharge after ileostomy. It acts best in an alkaline medium and only on the protein moiety of the mucoproteins, since no reducing substances are produced. It also digests azocasein and gelatin. It does not appear to be trypsin or erepsin.

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Components of Grass Pollen Extracts in Relation to Skin Reactivity in Allergic Subjects. By Rosa Augustin.* (*Wright-Fleming Institute, St Mary's Hospital, London, W.* 2)

For nearly half a century crude grass pollen extracts have been used in the treatment of hay fever, but little is known about their chemical composition. Paper chromatography enabled us to demonstrate free amino-acids, peptides, carbohydrates, proteins and a number of pigments. Apart from a stationary 'base-line' fraction all the components proved biologically inactive when butanol/acetic acid mixtures were used as developing fluids.

Controversial reports have appeared with respect to the dialysability of the active components of grass pollens (Ungar, Cromwell & Moore, 1931; Stull, Cooke & Chobot, 1932; and Harley, 1937). All our own dialysing experiments, using a variety of techniques, indicated that the active substances are non-diffusible through semi-permeable membranes. This was confirmed by ultrafiltration and Gradocol membrane experiments. A molecular weight $>10\,000$ had therefore to be assumed as opposed to the 5000 or less generally accepted (Abramson, 1951). Diffusion and ultracentrifugal studies on our active material were kindly carried out by Dr P. Charlwood of the Middlesex Hospital. He found a molecular weight of 13 900. Paper chromatography on intact sac contents from dialyses, or on residues from ultrafiltration, gave a 'base-line' spot only on spraying with ninhydrin.

Contrary to previous reports reviewed recently (Augustin-Friedmann, 1952) it has been possible to produce readily and regularly precipitating antisera to the active components of the extracts in rabbits and guinea pigs.

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Chemical, Immunological and Electrophoretic Data of Biologically Active Protein Components of Grass Pollen Extracts. By Rosa Augustin.* (*Wright-Fleming Institute, St Mary's Hospital, London, W.* 2)

Paper chromatography of the active base-line spots described in the previous communication revealed seventeen of the common amino-acids (lysine, arginine, aspartic acid, cystine, glutamic acid, histidine, glycine, serine, threonine, alanine, proline, valine, methionine, tyrosine, tryptophane, phenylalanine, leucine) as well as γ-amino-butyric acid and carbohydrates, thus proving the activity to be of protein nature.

Fractionation into globulins and albumins was possible by means of ammonium sulphate. Both fractions proved active on skin testing in allergic patients, but only the globulin fraction precipitated readily with rabbit or guinea-pig antisera produced to dialysed extract residue. The inactivity of Harley's pollen globulins was most probably due to his method of isolation which included heat treatment (Harley, 1937).

Isoelectric precipitation followed by NaCl fractionation made it possible to separate a strongly pigmented carbohydrate-rich fraction from almost colourless carbohydrate-poor fractions. All the fractions proved about equally active on an equal weight basis, and all yielded on hydrolysis the same amino-acids as had already been found in the unfractionated residue ('base-line' spot). The composition of the carbohydrate component appeared to be also the same in all the fractions.

In 1947, Abramson claimed to have isolated by electrophoresis the main active principle of timothy

* In receipt of a full-time research grant from the Asthma Research Council.

grass pollen as a practically stationary colourless fraction which he termed 'pratensin'. Evidence will be presented to show that this 'pratensin' appears to be an artifact due to salt interference in his undialysed extract. Our carbohydrate-rich pigmented fraction showed very high electrophoretic mobility at pH 7·4. Under the same conditions a little less fast-moving identical main peaks could be demonstrated in the four NaCl fractions. There were some minor impurities.

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The Decarboxylation of Valine and Leucine by Washed Suspensions of *Proteus vulgaris*. By H. K. King. (*Biochemistry Department, Johnston Laboratories, University of Liverpool*)

Washed suspensions of *Proteus vulgaris* decarboxylate L-valine and L-leucine with production of the corresponding amines, which were identified by distillation at alkaline pH and comparison with authentic samples on paper chromatograms run with butanol-acetic acid and phenol-water. This accords with the finding of these amines by Proom & Woiwod (1951) in culture filtrates of this organism when the appropriate amino-acid was sole nitrogen source. Decarboxylation is optimal at neutral pH, with $Q_{CO_2} = 20$ for valine. The highest Q values are obtained with cells grown at neutral pH and harvested before the end of the logarithmic phase of growth. Organisms grown on a medium with lactate as sole carbon source (plus a trace of nicotinamide, a growth factor for this organism) show no activity. Addition of valine or leucine to the medium gives cells active towards *both* amino-acids, suggesting that a single enzyme is involved. Cell-free extracts obtained by mechanical disintegration (but not from acetone powders) were fully active. The Michaelis affinity constant is high (0·2 M for valine).

The valine/leucine decarboxylase thus differs strikingly from the classical bacterial decarboxylases described by Gale (1946). These acted only on amino-acids with more than two polar groups, had strongly acid pH optima, were formed only in cells grown at acid pH, and were most active in mature cultures.

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The Effect of Reduced Temperature on Swelling and Electrolyte-accumulation in Tissue Slices. By H. Aebi (introduced by R. E. Davies). (*'Hallerianum' and Medizinisch-chemisches Institut der Universität, Berne, Switzerland*)

Under suitable conditions guinea-pig liver slices maintain their original water content as well as their potassium concentration gradient between tissue and suspending medium. On the other hand, swelling and leakage of potassium from the sliced tissue occurs in the cold, in anoxia and in a medium free of calcium or containing less than 10 millimolar potassium ions (Aebi, 1950, 1952).

Reduction of the incubation temperature to 22° or 4° causes a gradual decrease of the K gradient between liver slices and the isotonic medium. The average K concentration in the fresh tissue is 119 mM (mmoles/kg. tissue water); after incubation a decrease to 105 (37°), 55 (22°) and 24 mM (4°) is observed, the suspending medium containing 11 mM-K throughout. At the same time the tissue volume increases to 1·10 (37°), 1·21 (22°) and 1·50 (4°). Rabbit-brain cortex slices behave in a similar manner as to K gradient; in contrast, the swelling of the slices is reduced when the temperature is lowered, the tissue volume being 1·92 (37°), 1·55 (22°) and 1·06 (4°).

In rabbit-kidney cortex slices, however, the 'critical temperature', which is necessary to maintain the original K gradient, seems to lie below 22°. After incubation at either 37° or 22° the original K content of 100 mM even rises up to 130 mM, and incubated at 4° only drops to 69 mM; simultaneously the tissue volume changes from 0·92 (37° and 22°) to 1·09 (4°). In contrast to liver slices, which rapidly give off all their K in the cold, kidney-cortex slices hardly lose more than half of their original K content even after a several hours' leaching period at 4° (cf. Mudge 1951).

The sum of K and Na in the slices behaves in the following manner: Owing to accumulation the original value (range for different organs: 160–195 mmoles/kg. tissue water) is increased after incubation, the effect being less pronounced or even being absent at lower temperatures; e.g. guinea-pig liver slices (value for fresh tissue 172 mM) accumulate to 237 mM at 37°, to 214 mM at 22° and to 180 mM at 4°. Even higher values are observed in rabbit-kidney cortex slices: 260 mM at 37° and 22°, 218 mM at 4° (cf. Robinson 1952). Almost no accumulation of Na+K can be seen in rabbit-brain cortex slices (165–172 mM) at any temperature.

The fact that in sliced kidney cortex and liver a certain temperature is needed for prevention of swelling and accumulation of K and (Na+K) indicates that these processes are linked up with metabolic activity (cf. Davies & Whittam, 1953). Brain-cortex slices also take in K and Na at 37°, but cannot concentrate them, presumably owing to an insufficient expulsion of water.

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The Source of Antibody in Rabbit Milk. By P. N. Campbell, J. H. Humphrey and T. S. Work.
(*National Institute for Medical Research, Mill Hill, London*)

It was shown by Campbell & Work (1952) that in the rabbit the radioactivity of the milk proteins after intravenous injection of radioactive amino-acids was initially very much greater than that in the plasma proteins. This suggested that most of the milk proteins were synthesized in the mammary gland from free amino-acids rather than from plasma protein. The results did not exclude the possibility that some milk protein was derived, directly or indirectly, from plasma protein. The occurrence of antibody in the milk of immunized animals provides a method for testing this possibility.

A lactating rabbit was immunized by intravenous injection of formolized *Pneumococcus* type III until antibody was present in the milk in sufficient quantity for isolation. It was found that the concentration of antibody in the milk was 5–10% of that in the plasma. Three days after the last injection of antigen, approximately 25 μc. (6·7 mg.) ^{35}S-DL-methionine was injected intravenously, and milk was collected 6 hr. later. A blood sample was taken at this time. Milking and bleeding were repeated 24 hr. later. Fat and most of the casein in the milk were removed by repeated high-speed centrifugation in the cold, after which antibody was precipitated by addition of type-specific *Pneumococcus* polysaccharide at optimal proportions. Blood-serum antibody was isolated in a similar manner. The antibody-antigen precipitates, together with the casein, the residual whey proteins, the serum albumin fraction and the residual γ-globulin, were washed and dried and their radioactivity determined. The activities 6 hr. after injection (c./min./0·3 cm.^{2} samples of infinite thickness) were as follows: serum antibody 16, milk antibody 7, casein 108, whey protein 91, serum globulin 17, serum albumin 18. Thirty hours after injection the corresponding activities were 17, 17, 30, 38, 16 and 13.

These results show that there is a direct transfer of antibody from blood plasma to milk.

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Thermodynamics of Acid Dissociations. By J. H. Ashby, E. M. Crook and S. P. Datta.
(*Department of Biochemistry, University College, London*)

The thermodynamic pK's and the thermodynamic quantities associated with the first and second dissociations of β-glycerophosphate and the second dissociations of glucose-1-phosphate and succinic acid have been measured by the method of Harned & Ehlers (1932). The measurements were carried out in solutions containing chloride, at 5° intervals between 5° and 50° using hydrogen electrodes in cells without liquid junction with silver-silver chloride reference electrodes. The values of the

thermodynamic pK's at 25° are 1·335, 6·650, 6·502 and 5·661 respectively. The accuracy is approximately ±0·003 pK units.

The variation of each pK with temperature is accurately represented by an equation:

$$pK = \frac{A}{T} - D + CT,$$

where A, D and C are constants and T is the absolute temperature. The values of the constants are 891·9, 13·48 and 0·0284 for pK_1 of β-glycerophosphate; 1667·4, 4·84 and 0·0198 for pK_2 of β-glycerophosphate; and 1489·6, 3·81 and 0·0178 for pK_2 of glucose-1-phosphate. The data for succinic acid are not yet available.

From the variation of pK with temperature can be calculated the standard change of free energy, heat content, entropy and heat capacity due to the ionization. Examples of the value of these quantities and a brief description of the method will be given.

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Metabolic Exchange of the Sulphate Group in Mucopolysaccharides. By H. Boström and E. Jorpes. (*Chemistry Department II, Caroline Institute, Stockholm*)

The half-life time of the sulphate group in chondroitin sulphuric acid in the cartilage of rats is about 16 days, in the subcutaneous tissue somewhat shorter. ^{35}S-labelled sodium sulphate is built into the chondroitin sulphuric acid and also into thin slices of cartilage suspended in a nutrition medium. The esterification process behaves like an ordinary enzyme reaction. Judging from the inhibition of the reaction an SH enzyme seems to be an essential component. The reaction is strongly inhibited by cortisone.

The incorporation and elimination of the ester sulphate group in animal mucopolysaccharides can also be followed by means of autoradiography. The density of the autoradiographs corresponds quantitatively to the intensity of the radiation of the sulphate group of the chemically isolated chondroitin sulphuric acid. The autoradiographic technique thus allows quantitative studies even where material is not available for chemical isolation of the corresponding sulphate mucopolysaccharides. Furthermore, it gives a good picture of the distribution of these compounds in the body.

Identity of the Natural 'co-factor' of Bracken Thiaminase. By J. E. Watkin, A. J. Thomas and W. C. Evans. (*Department of Agricultural Chemistry, University College of North Wales, Bangor*)

The thermolabile system of bracken which splits aneurin according to the following equation:

$$Pm.CH_2.Th + H_2O \rightarrow Pm.CH_2.OH + Th + H^+$$

(Pm = 2-methyl-4-aminopyrimidine;
Th = 4-methyl-5-hydroxyethyl thiazole)

had previously been separated into thermostable dialysable 'co-factor', and thermolabile non-dialysable 'apo-enzyme' components (Evans, Jones & Evans, 1950; Evans & Jones, 1952). About 90% of the aneurin destruction caused by neutral aqueous bracken extracts is due to the above system; the residual 10% activity (to the thiochrome test) is non-enzymic. This latter factor may be removed quantitatively from such extracts by adsorption on charcoal. The chemical identity of this thermostable factor is as yet unknown. The plant thiaminase appears to be solely responsible for the production of avitaminosis B_1 in rats when bracken is included in an otherwise complete diet, since steamed bracken fails to produce the symptoms.

The apo-enzyme may be purified and preserved in the freeze-dried state by the following procedures: isoelectric precipitation; solvent precipitation, e.g. with ethanol and acetone; fractional precipitation with ammonium sulphate, all in the cold.

Several meta-substituted aromatic primary amines are able to act as artificial 'co-factors' to the apo-enzyme of bracken, a behaviour paralleled by the seemingly analogous enzyme system present in carp, clams, and *Bacillus thiaminolyticus* (Sealock & Davis, 1949; Evans & Jones, 1952; Fujita & Tashiro, 1952.)

The identity of the naturally occurring 'co-factors' has not been reported from any of the tissues known to contain thiaminase. Bracken thiaminase co-factor activity is widely distributed, being present in extracts of plant and animal

origin, e.g. yeast, liver, urine, grass, etc. Our most active preparations from the bracken plant contain appreciable amounts of several amino-acids, including proline. Of these, pure DL-proline and L-hydroxyproline are active as 'co-factors' in the *in vitro* bracken thiaminase system. Related substances, pyrrolidine and sarcosine show a comparable activity, whilst piperidine-2-carboxylic acid, piperidine, taurine, *b*-alanine, and glycyl-glycine are all active, but to a lesser degree. These compounds show a parallel behaviour in the carp and clam thiaminase systems.

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Linkage of Na and K Transport as a Basis for the Interpretation of the Kinetics of Movement of these Ions between Human Erythrocytes and the Suspension Medium. By E. J. HARRIS. (*University College, London*)

Many of the facts concerning the rates of penetration of the human erythrocytes by alkali ions can be interpreted on the following basis. (*a*) A passive flux of Na and K ions is present across the cell membrane in each direction in amount determined by the product of the respective concentration, permeability constant, and a pH-dependent factor determined by the electrical asymmetry. This latter factor is equal to $1/f$ for efflux and to f for the influx with $f^2 = Cl_e/Cl_i$ (cf. Harris & Maizels, 1951, 1952). Thus, passive Na influx $= fk_1[Na_e]$ and passive Na efflux $= k_1[Na_i]/f$ and similarly for K, but the k_1 applying to K ions is lower (50–70 %) than the one applying to Na ions. (*b*) An active metabolically potentiated efflux of Na takes place at rate proportional to $[Na_i]$ provided conditions are suitable. A prerequisite for active Na efflux is the presence of K in the external medium. The Na carriers having reached the exterior of the cell are assumed to be converted to K carriers which take in K ions in amount very close to half the number of Na ions carried out, and are reconverted in the cell interior to Na carriers. As active Na efflux and K influx are independent of external K when the latter exceeds 1–2 mequiv./l. it seems that the inward-bound carriers find an adequate supply of K ions adsorbed on the cell surface so that frequency of collision of K ions is not a factor limiting the rate of penetration.

In the steady state K exchanges with a low rate constant (0·016 hr.$^{-1}$ at 37°) which is characteristic of passive transfer, while sodium exchanges with a high rate constant (0·3 hr.$^{-1}$ or more) which is largely due to the active process. When cells gain or lose K at 37° both rate constants are involved (Ponder, 1951). The activation energy of the passive fluxes appears to be equal to 14 000 cal./mole, whereas the value applying to the active components of the K influx and Na efflux is nearly twice as great. Values in the literature are not derived from separate consideration of active and passive fluxes. The temperature dependence of the rate of penetration will in general differ according to whether the cells are in a steady state or not.

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The Excretion of Kynurenine and 3-Hydroxykynurenine by Man. By C. E. DALGLIESH and S. TEKMAN. (*Postgraduate Medical School, Ducane Road, London, W.* 12)

Makino, Satoh, Fujiki & Kawaguchi (1952) have reported that 3-hydroxykynurenine is responsible for the diazo reaction and the Weiss urochromogen reaction shown by the urine of acute tuberculous patients. To confirm this, urines of various tuberculous patients have been examined by the methods of Dalgliesh (1952). Of the urines examined one was found to contain L-3-hydroxykynurenine. Besides having more severe tuberculosis this patient also had a higher temperature. The urines were then examined of many patients having a variety of disorders (influenza, bronchitis, etc.) and having a temperature greater than 100° F. Some urines (always deep in colour) were found to give a positive diazo reaction and these urines also contained L-3-hydroxykynurenine. A few urines

showing a negative diazo reaction were examined and these contained no detectable hydroxykynurenine. Patients excreting a large amount of hydroxykynurenine also excreted a much smaller amount of L-kynurenine, whereas in the urines of those patients excreting only small amounts of hydroxykynurenine no kynurenine was detected. The excretion of hydroxykynurenine by the human is therefore unrelated to tuberculosis. The amount excreted in some cases seems much too large to have been derived from dietary tryptophan. The excretion is therefore considered to indicate a rapid breakdown of body protein due to the high metabolic rate in fever. Thyrotoxic patients so far examined (having a raised metabolic rate but no fever) have shown no hydroxykynurenine excretion.

When 10 g. DL-tryptophan was taken by mouth by the normal human kynurenine was excreted (both L and D-isomers, the amount of the L-isomer being greater) but no hydroxykynurenine. The excretion of kynurenine and hydroxykynurenine is due to their formation at a greater rate than their breakdown by the enzyme kynureninase (Knox, 1953). As the same enzyme probably attacks both substances the reason is not clear why excess tryptophan should give rise to kynurenine in normal humans, but to hydroxykynurenine in cases of fever. Possible explanations are (*a*) that the enzymes involved are markedly temperature-dependent, e.g. rise in temperature may increase the rate of the kynurenine-hydroxykynurenine conversion much more than it increases the rate of kynurenine formation, or (*b*) an alternative pathway to hydroxykynurenine may exist which plays a greater part in the metabolism of endogenous rather than exogenous tryptophan, or which plays a greater part at higher temperatures. Such a pathway could be via 7-hydroxytryptophan.

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Variation in the Organic Content of Deciduous Enamel and Dentine. By M. V. STACK. (*Department of Dental Medicine, Guy's Hospital, London, S.E.* 1)

Bird, French, Woodside, Morrison & Hodge (1940) have reported the organic content of deciduous enamel to be 0·7–12 %, measuring the loss of weight after extraction with hot alkaline ethylene glycol. This range is very much wider than the 0·4–0·8 % found for permanent enamel by digestion with a dichromate-sulphuric acid reagent (Stack & Williams, 1952). The range of 5–42 % given by Bird *et al.* for the organic content of deciduous dentine also greatly exceeds that for the total nitrogen content of permanent dentine (2·9–3·9 %) as observed by Stack (1951).

Enamel and dentine were separated from the pulverized crowns of a similar representative selection of fifty deciduous teeth, using the procedure developed for permanent teeth. The variable degree of root resorption, and slight damage to the enamel of half the teeth, made sampling from the whole of both tissues impossible. A representative group of crowns from five permanent teeth was submitted to the same procedure before and after studying the series of deciduous teeth, in order to compare absolute values as well as variations.

Every value for the organic content of deciduous enamel fell within the range 0·5–0·9 %, after correction for the $0{\cdot}23 \pm 0{\cdot}1$ % dentine remaining in each sample. The mean of 0·7 % was significantly different from the mean of 0·6 % found for permanent enamel using this technique. These means were, in both cases, eight times greater than the standard deviations, which were corrected for analytical errors. The ranges found by this direct analysis of the organic content of enamel were predicted from the specific gravity ranges of 2·86–2·98 for deciduous enamel (Berghash & Hodge, 1940) and 2·89–3·00 for permanent enamel (Manly, Hodge & Ange, 1939). The dentine organic content, also measured by digestion with the dichromate-sulphuric acid reagent, was 15–18 %, the mean being not significantly different from that for permanent crown dentine (ten samples). The corrected coefficients of variation were 4·1 % for the organic content of deciduous dentine and 4·4 % for the nitrogen content of permanent dentine. There was no correlation between the organic contents of the enamel and dentine, nor between either of these and age, sex or tooth location.

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Acid-labile Precursors of Naphthalene produced during Metabolism of Naphthalene by Rats and Rabbits. By E. BOYLAND and J. B. SOLOMON. (*Chester Beatty Research Institute, The Royal Cancer Hospital, London, S.W.* 3)

Baumann & Herter (1877) noticed that when the acidified urine of rabbits dosed with naphthalene was steam distilled crystals of naphthalene separated in the distillate. Bourne & Young (1934) suggested that this might be formed by the dehydration of 1:2-dihydro-2-naphthol; this substance, however, has never been isolated from urines either by ether extraction or steam distillation. Chang & Young (1943) found that this naphthalene precursor accounted for about 16 % of naphthalene fed to rats and that hydrocarbon precursors were also excreted by rats dosed with anthracene and phenanthrene.

Similar acid-labile hydrocarbon precursors are produced during the metabolism of tetralin and 1:2-dihydronaphthalene (Pohl & Rawicz, 1919). Tetralin was converted into *ac*-1-tetralol glucuronide in dogs and *ac*-2-tetralol glucuronide in rats and rabbits. When these compounds were steam distilled from acid solution 1:2-dihydronaphthalene and some naphthalene were produced. 1:2-Dihydronaphthalene was converted by the rabbit into a substance which on similar acid treatment yielded naphthalene; Pohl and Rawicz suggested that this may be formed from the decomposition of a 1:2-dihydronaphthol glucuronide. It seemed possible that a glucuronide of this type might be produced during the metabolism of naphthalene.

The urines of rats and rabbits which had been dosed with naphthalene were percolated through a charcoal column which was then eluted with water. The aqueous eluate contained the naphthalene precursor and glucuronide. The 1:2-dihydroxy-1:2-dihydronaphthalene glucuronide was then eluted with 50 % aqueous acetone. As no water-insoluble salt of the naphthalene precursor could be formed the gum obtained on evaporation of the aqueous eluate was dissolved in methyl alcohol, methylated with diazomethane and then acetylated. A crystalline compound was obtained corresponding to that of a triacetyl derivative of 1:2-dihydronaphthol glucuronide methyl ester. Found: C 59·9, H 6·1. $C_{23}H_{26}O_{10}$ requires C 59·7, H 5·7 %. Both derivatives from rat and rabbit urines melted at 203° C.; rabbit $[\alpha]_D^{20^\circ} = +23^\circ$: rat $[\alpha]_D^{20^\circ} = -84^\circ$ ($c = 1$ % in chloroform).

Treatment of these derivatives with cold dilute acid yielded naphthalene. The ultraviolet-absorption spectra indicate a 1:2-dihydronaphthalene structure. When the aqueous eluate was incubated with β-glucuronidase a steam volatile oil was liberated which on steam distillation from acid yielded naphthalene.

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The Vitamin E Activity of Substances Related to Methylene Blue. By T. MOORE, I. M. SHARMAN and R. J. WARD. (*Dunn Nutritional Laboratory, University of Cambridge and Medical Research Council*)

In a previous communication (Moore, Sharman & Ward, 1953) we have reported our failure to confirm that methylene blue can replace vitamin E in preventing the resorption of foetuses in the rat, but our success in using it to prevent certain other manifestations of vitamin E deficiency, including brown discoloration of the rat's uterus. We have now tested other redox dyes and anti-oxidants for their ability to prevent brown uterus in rats kept for about 4 months upon a diet deficient in vitamin E. Discoloration was prevented by inclusion in the diet of 0·068 % thiodiphenylamine, which could be derived from methylene blue by the removal of both its methylated amino groups. Bindschedler's green, which could be derived from methylene blue by the removal of its sulphur atom, was also effective when given as 0·126 % of the diet. No protection was found, however, at the doses given by other dyes related to methylene blue, such as thionine (0·089 %) and methylene violet (0·087 %), by phenylene blue (0·067 %) which is related to Bindschedler's green, or by other redox substances such as neutral red (0·126 %), 2:6 dichlorophenolindophenol (0·126 %), Janus green (0·126 %) and rosinduline (0·126 %). Other substances found ineffective were tetraethyl thiuram disulphide (Antabuse; 0·025 %) nordihydroguairetic acid (0·5 %) and *p*-phenylene diamine (0·037 %).

Various derivatives of hydroquinone including the monomethyl, dimethyl and diethyl ethers, and 2, 5-*tert.*-butyl hydroquinone, octyl hydroquinone and toluhydroquinone also had no effect when given in oily solution, separate from the diet, in daily doses of 1 mg.

Definite conclusions as to details of chemical structure which are necessary to give activity in protecting against brown uterus must await trials with further substances, and with some of those already tested at different levels of dosing. For the present, however, it seems reasonable to conclude there is some degree of specificity in the requirements necessary for activity, and that it is not a general property shared by a wide range of redox dyestuffs and anti-oxidants.

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DEMONSTRATIONS

Apparatus and Methods for the Determination of Thermodynamic Dissociation Constants of Acids. By J. H. Ashby, H. B. Clarke, E. M. Crook, D. C. Cusworth and S. P. Datta. (*Department of Biochemistry, University College, London*)

A Chromatographic Investigation of Taurine Metabolism in Normal and Pyridoxine Deficient Rats. By H. Blaschko, S. P. Datta and H. Harris. (*Department of Pharmacology, Oxford, and Department of Biochemistry and the Galton Laboratory, University College, London*)

Taurine is known to be present in the urine of normal rats (Datta & Harris, 1951), and it is known that rat liver contains an enzyme which decarboxylates L-cysteic acid presumably forming taurine (Blaschko, 1942). Chromatograms are shown which demonstrate that the enzymic decarboxylation of L-cysteic acid results in the production of taurine. Rat livers were dispersed with an equal weight of 0·67 M-phosphate buffer pH 7·4 at 0°. 1·8 ml. of the dispersion was incubated anaerobically in Warburg flasks with 0·2 ml. neutralized 0·1 M-L-cysteic acid. The reaction was stopped by the addition of 2 ml. 20 % trichloroacetic acid, the protein was removed by centrifugation and chromatograms were run in phenol and collidine-lutidine with 10 μl. of the supernatant. Controls were run by incubating liver dispersion with water and L-cysteic acid with buffer. The chromatogram of the full system at zero time shows only cysteic acid, while that at 270 min. shows a large spot due to taurine and only a trace of cysteic acid, there are, however, spots due to glycine, glutamic and aspartic and other amino-acids. The chromatogram of the liver dispersion incubated alone shows glycine, glutamic and aspartic and traces of other amino-acids but no taurine or cysteic acid; it is inferred that these other amino-acids are products of liver protein autolysis.

The L-cysteic acid decarboxylase activity of rat's liver is diminished or absent in pyridoxine deficient animals (Blaschko, Carter, O'Brien & Sloane Stanley, 1948); we therefore examined the urine of such deficient animals. The urines of nineteen control animals on the deficient diet supplemented with pyridoxine all showed taurine in the chromatograms, while in the urines of thirty-seven deficient animals taurine was much less regularly present, and the frequency of its occurrence was least in animals which had been longest on the deficient diet. In all but three of the thirty-seven deficient animals the urines contained relatively large amounts of a ninhydrin-reacting substance which behaves chromatographically as ethanolamine phosphate.

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FORTHCOMING PAPERS

It is hoped to publish the following papers in the next issue of the *Biochemical Journal*:

The isolation of pregnane-3α:17α:20α-triol from the urine of normal men. By R. I. Cox and G. F. Marrian

Oxidation through the cytochrome system of substituted phenylenediamines. By H. G. Borei and U. Björklund

Chemistry of insect cuticle:

1. The water-soluble proteins. By R. H. Hackman
2. The water-insoluble proteins of the cuticle of *Aphodius howitti* Hope (Coleoptera). By R. H. Hackman
3. Hardening and darkening of the cuticle. By R. H. Hackman

The role of sulphydryl groups of yeast carboxylase. By A. O. M. Stoppani, A. S. Actis, J. O. Deferrari and E. L. Gonzalez

Studies in detoxication. 50. The isolation of methyl and ethyl glucuronides from the urine of rabbits receiving methanol and ethanol. By I. A. Kamil, J. N. Smith and R. T. Williams

Preparation of uricase. By E. Leone

A method for measuring the deposition of ^{32}P in phosphatidylethanolamine and its application to rat-brain tissue. By J. M. Norman and R. M. C. Dawson

Changes in body composition and respiratory quotient of adult female rats treated with purified growth hormone. By A. L. Greenbaum

The mobilization of lipid by anterior pituitary growth hormone. By A. L. Greenbaum and Patricia McLean

The influence of pituitary growth hormone on the catabolism of fat. By A. L. Greenbaum and Patricia McLean

Excretion of formate after methanol ingestion in man. By L. P. Kendal and A. N. Ramanathan

A cholinesterase inhibitor derived from *OO*-diethyl *O*-*p*-nitrophenol thiophosphate *in vivo*. By J. C. Gage

The assimilation of amino-acids by micro-organisms. 16. Changes in sodium and potassium accompanying the accumulation of glutamic acid or lysine by bacteria and yeast. By R. Davies, Joan P. Folkes, E. F. Gale and Lois C. Bigger

A new method for preparing flavin-adenine dinucleotide. By L. G. Whitby

The inhibition of erythrocyte cholinesterase by tri-esters of phosphoric acid. 3. The nature of the inhibitory process. By W. N. Aldridge

The application of counter-current methods to the fractionation of lipid material from brain. By P. G. Cole, G. H. Lathe and C. R. J. Ruthven

A nomogram for ammonium sulphate solutions. By M. Dixon

Examination of some components of cephalopod and sperm-whale liver oils by the chromatographic method. By R. M. Brachi

Appendix—Examination of salps. By R. M. Brachi

Phospholipins. 1. Partition chromatography of egg-yolk phospholipins on cellulose. By C. H. Lea and D. N. Rhodes

The polarographic estimation of steroid hormones. 6. Determination of individual adrenocortical hormones in human peripheral blood. By C. J. O. R. Morris and D. C. Williams

Lipovitellin:

3. The effect of sugars on the stability of freeze-dried lipovitellin. By J. C. Hawke and C. H. Lea
4. Chemical composition of the lipid and of the fractions removed by various treatments. By J. C. Hawke and C. H. Lea

Etherification of noradrenaline in acidified 95% ethanol. dl-2-Ethoxy-2-(3′:4′-dihydroxyphenyl)ethylamine hydrochloride. By I. Serlin and M. Goldenberg

[P.T.O.]

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the authors.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **317th Meeting** *of the Biochemical Society was a joint meeting with the Société Belge de Biochimie and was held in the Faculty of Medicine, Brussels University, 115 Boulevard de Waterloo, Brussels, at 9.0 a.m. on Saturday, 11 April 1953, when the following papers were read.*

COMMUNICATIONS

A Simple Method for Preparing Crystalline Rennin. By N. J. Berridge and Cora Woodward. (*National Institute for Research in Dairying, University of Reading*)

The preparation of crystalline rennin previously described (Berridge, 1945) was somewhat long and inconvenient, but the knowledge gained about the properties of the crystals suggested that a simpler method might be successful. Since then Mogensen (1951) has shown that the mere precipitation of commercial rennet with saturated sodium chloride gives a product with proteolytic properties resembling those of the crystalline material, and with considerably enhanced milk-clotting activity per unit of dry weight. Some years previously Hankinson (1943) had also achieved considerable purification by these means.

Rennin has now been crystallized several times by the following procedure or minor modifications of it. One gallon (5·4 l.) of commercial rennet (from Bengers Ltd., Holmes Chapel, Cheshire) was saturated with sodium chloride. The supernatant liquid was decanted on to large fluted filters made from Whatman no. 3 paper supported at the tip with a small no. 54 paper. The first opalescent runnings were returned to the filters. Four days were required for the filtration. After this time the papers had become partly dried, so that they were impregnated with solid sodium chloride. Moreover, the precipitated proteins were entrained in the filters and could not be removed by scraping. The no. 3 papers were therefore pulped and extracted exhaustively with distilled water. This required four extractions of approximately 250 ml. each. The combined extracts were filtered (Whatman no. 1), adjusted to pH 5·4 and again saturated with sodium chloride added through a rotating, semi-permeable membrane (Visking tubing) as suggested by McMeekin (1939).

Owing to the slow addition of the salt the protein formed a granular precipitate which was easily centrifuged down. It was redissolved in 60 ml. of distilled water, and on standing overnight in the refrigerator gave a good crop of crystals with a yield of 18 % of the activity of the starting material.

The crystals first obtained may be dissolved by dialysis against 0·1 % sodium chloride as mentioned by Schwander, Zahler & Nitschmann (1952). Amorphous rennin precipitated from such a solution by saturation with sodium chloride will recrystallize when dissolved in 0·1 N-acetate buffer at pH 5·4.

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Inhibition of the Cholinesterase of the Central Nervous System by Organophosphorus Compounds. By A. N. Davison. (*Toxicology Research Unit, Serum Research Institute, Woodmansterne Road, Carshalton, Surrey*)

Recently, certain organophosphorus compounds have been shown to cause demyelination of peripheral nerve and of certain tracts of the spinal cord of chickens (Earl & Thompson, 1952; Barnes & Denz, 1953). Earl & Thompson (1952) found that tri*ortho*-cresyl-phosphate (TOCP) inhibited pseudocholinesterase of plasma of the central nervous system in chickens and showed that the enzyme activity remained low until after paralysis first appeared (10–12 days). These observations led Earl & Thompson to suggest that pseudo-cholinesterase may be concerned in myelin metabolism and therefore its inhibition may result in demyelination.

A number of organophosphorus compounds, all selective inhibitors of pseudo-cholinesterase *in vitro*, were injected into chickens. Three of these compounds (TOCP, di-isopropyl fluorophosphonate (DFP) and *bis*(mono-isopropylamino) fluorophosphine oxide (Mipafox, Isopestox)) caused

demyelination and three did not (*o.o*.di-isopropyl *o.p*-nitrophenyl phosphate (iso-E 600), tetra-isopropyl pyrophosphate (TIPP) and tetra (mono-isopropyl) pyrophosphoramide (iso-Ompa)). True and pseudo-cholinesterase levels of the central nervous system and plasma have been determined at various times after injection (Davison, 1953). The findings (Earl & Thompson, 1952) of prolonged inhibition of pseudo-cholinesterase after administration of TOCP have been confirmed. Initially, all the other compounds powerfully inhibited pseudo-cholinesterase, but recovery of enzyme activity was rapid (about 50 % in 2 days and 100 % in 12 days). The recovery pattern was similar for compounds which produced demyelination and those that did not. TOCP and iso-Ompa were found to inhibit true cholinesterase only slightly, but the other substances inhibited the enzyme by about 95 %; again the recovery pattern was rapid and similar for all the compounds.

DFP was injected into rats, and although pseudo-cholinesterase of brain and spinal cord was completely inhibited, paralysis did not result and demyelination was not detected on histological examination. Myers & Mendel (1952) have also found no evidence of demyelination even after prolonged and complete inhibition of rat brain and spinal cord pseudo-cholinesterase with TOCP.

These observations show that prolonged inhibition of pseudo-cholinesterase is not an essential prerequisite of demyelination. The relationship between inhibition of pseudo-cholinesterase and demyelination remains obscure; nevertheless, only compounds which are known selective inhibitors of pseudo-cholinesterase have produced paralysis.

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Association of the Penicillin-binding Component with a Lipid Fraction in *Staphylococcus aureus*. By P. D. Cooper. (*Wright-Fleming Institute, St Mary's Hospital Medical School, Paddington, W. 2*)

Rowley, Roberts, Cooper & Lester Smith (1950) showed that penicillin-sensitive organisms contained a component (PBC) which bound penicillin strongly, and which appeared related to its antibacterial activity. Most of this PBC appeared in the cell-wall fraction prepared by mechanical rupture in a formalin/thioglycollate/citrate mixture ('medium A', Few, Cooper & Rowley, 1952), but was removed from the cell walls or destroyed when shaken in distilled water alone (Cooper, Rowley & Dawson, 1949).

The difference in colour observed between cell walls prepared in distilled water (white) and those from medium A (yellow) suggested that the latter may contain considerable lipid matter (staphylococcal pigments are carotenoid in character, Sobin & Stahly, 1942).

This was confirmed by analysis, and the proportion of lipid phosphorus in cell wall, 'lipid particle' (Mitchell & Moyle, 1951) and supernatant fractions closely followed the proportion of PBC when prepared in distilled water or in medium A.

The distribution of ^{35}S from organisms previously treated with radiopenicillin was also similar to that of the lipid P. The presence of formalin/citrate/thioglycollate appeared to allow the cell walls to retain the bulk of the lipid P, so that the lipid particles, normally 5–6 % of the dry weight of the organisms, were no longer formed. Cell walls prepared from distilled water comprised 20–25 % of the dry weight of the intact cells, and cell walls from medium A 30–40 %.

The 'lipid particles', which appeared as an orange waxy sediment by spinning at 12 000 g. after removal of cell walls, specifically bound 13 units penicillin per g. dry wt., compared with 1·5 units/g. for intact cells and 4·5 units/g. for cell walls from medium A. Lipid solvents removed most of the ^{35}S from radiopenicillin pre-treated cells, and removed or destroyed most of the PBC.

Each ml. of the clear supernatants from high-speed centrifuging after rupture of organisms at 25 mg./ml. dry wt. contains enough PBC to neutralize 0·02 unit of penicillin, detectable by a very sensitive plate assay of penicillin. Thus the presence of PBC in the lipid particle and supernatant fractions shows that PBC is not destroyed on rupture, but only washed away from the cell walls.

Penicillin may thus be said to react with a lipid fraction which appears to be close to the cell wall in the intact cell. There is also good evidence that the intact cell may actually contain at least twice as much PBC as appears to be directly available for reaction with penicillin.

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Isolation and Properties of Histones from Thymus Nucleoproteins. By J. A. V. Butler, P. F. Davison and D. W. F. James. (*Chester Beatty Research Institute, London, S.W.* 3)

In view of the possibility that the acids normally used (e.g. Kossel, 1928) to extract histones from nuclear preparations and nucleoproteins might degrade them to some extent, we have experimented with alternative methods. Since calf-thymus nucleoprotein is largely dissociated in solutions of high ionic strength, it was thought that histones in an unmodified state could be prepared by precipitating the nucleic acid from such solutions by various agents. A number of solutions (NaCl, LiCl, $CaCl_2$) have been tried with methanol, ethanol, *n*- and *i*-propanol, acetone and lanthanum acetate as precipitants. The most effective agents are ethanol and acetone, but the nucleic acid precipitate is always contaminated with some protein, the amount depending mainly on the salt concentration. The less contaminated precipitates (N/P about 2·5) can be redissolved and on reprecipitation the N/P ratio reaches 1·9. Since the nucleoprotein contains up to 8% ribosenucleoprotein this is taken to indicate that all the protein initially attached to the desoxyribonucleic acid is left in solution.

The protein in this solution can be obtained by (*a*) salting out with ammonium sulphate or (*b*) dialysing out precipitant and salt. About half the protein becomes insoluble either on standing or during dialysis, probably owing to denaturation. It cannot be redissolved by any mild treatment. No qualitative difference of amino-acid content between the soluble and insoluble proteins was observed. The soluble part is electrophoretically complex, and according to sedimentation and diffusion experiments (made with the help of Dr K. Shooter) has a lower molecular weight than the acid-extracted histone. It therefore appears that the protein extracted under neutral salt conditions is degraded, probably by nuclear cathepsins (Maver, Greco, Løvtrup & Dalton, 1952). Attempts to prevent this degradation by adding a thiol inhibitor (ethyl iodoacetate), by buffering at pH 8 or by carrying out the preparation more rapidly appeared to decrease the proteolysis, but the products were always complex. It appears from this that acid extraction gives histones in a less modified condition. Studies of solubility as a function of solvent composition (Roche & Derrien, 1947) on both types of product confirmed the much greater homogeneity of the acid-extracted material. Only 80% of the protein, however, can be extracted in this way. Some chemical and other studies of the extracted material have been made.

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Crystalline Porphobilinogen from Urine of Rabbits Treated with Sedormid. By Phyllis E. Brockman and C. H. Gray. (*Department of Chemical Pathology, King's College, Hospital*)

We have previously shown (Brockman & Gray, 1953) that when crude solutions of porphobilinogen prepared from acute porphyria urines are heated, a porphyrin and a yellow pigment are formed in proportions depending on the acidity of the solution. When the porphobilinogen solution is allowed to stand at pH 1, a violet pigment is formed. Precisely similar behaviour is shown by crystalline porphobilinogen prepared from the urine of rabbits in which porphyria has been induced by administration of Sedormid (Schmid, 1952).

Rabbits were fed 250 mg. Sedormid/kg. body weight. After 10–12 days the urine may contain as much as 100 units (Vahlquist, 1939) of porphobilinogen/ml.

12 mg. of crystalline porphobilinogen (representing a 70% yield) were obtained from 360 ml. of a urine containing 6 units/ml., by the method of

Westall (1952), but using Deacidite FF instead of Dowex 2. The material obtained from rabbit urine contained 130 units porphobilinogen/mg. and resembled in every way that obtained by Westall from human porphyria urine. Elemental analysis gave C 52·67, H 6·90, N 11·52, O 28·91 in close agreement with that of Westall's material. On heating in 0·1–1·0N-HCl, a porphyrin was obtained in 36–40% yield. Chromatography of the free porphyrin in lutidine-H_2O (Nicholas & Rimington, 1951) revealed the presence of eight carboxyl groups. Chromatography of the methyl ester showed it to be uroporphyrin III, in agreement with the finding of Rimington (1953) that this is formed from crystalline porphobilinogen prepared from human material.

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The Calcium Content of Isolated Heart-Muscle Sarcosomes. By E. C. Slater and K. W. Cleland. (*Molteno Institute, University of Cambridge*)

It has been shown that the morphological changes and the inactivation of the α-ketoglutaric oxidase system which occur when heart-muscle sarcosomes (mitochondria) are exposed for brief periods to room temperature are associated with calcium contained in the sarcosomes and can be prevented by the addition of versene (Cleland & Slater, 1953; Slater & Cleland, 1952). By direct analysis using the method of Debney (1952), it has now been found that isolated sarcosomes contain all the calcium of heart muscle. However, it is probable that the calcium is not present in the sarcosomes in the intact heart muscle, but becomes bound during the isolation of the sarcosomes. (The heart muscle is ground by hand with sand or Pyrex glass beads in isotonic saline or sucrose and the sarcosomes separated from soluble proteins and from fibre fragments by differential centrifugation.) This is suggested by the fact that after a light grinding sufficient to liberate only about 30% of the sarcosomes (but probably all the sarcoplasm), the isolated sarcosomes contained approximately all the calcium of the heart muscle. Thus the sarcosomes retained in the fibres must have contained little calcium.

Sarcosomes isolated with isotonic saline or sucrose contained $0·099 \pm 0·006$ (standard error) μmole calcium/mg. sarcosomal protein. When a sample of these sarcosomes, containing 5·9 mg. protein and 0·49 μmole calcium, was suspended in 6 ml. of 0·16M-KCl, containing 10^{-4}M-$CaCl_2$, and the sarcosomes separated by centrifugation, the calcium content of the isolated sarcosomes was increased from 0·083 to 0·127 μmole/mg. protein, while half the calcium had been removed from the solution. Sarcosomes isolated with saline containing 0·01M-versene contained 0·014 μmole calcium/mg. protein. A preparation isolated with saline containing versene and then washed several times with versene-free saline contained 0·015 μmole calcium/mg. protein. When 10 mg. (protein) of this preparation were suspended in saline containing 10^{-4}M-$CaCl_2$, the sarcosomes reisolated by centrifugation contained all the calcium (the calcium content was increased to 0·068 μmole/mg. protein), while none was detectable in the supernatant.

Since the sarcosomal preparations used in these experiments were free of substrates, the uptake of calcium could not be due to active secretion of the type described by Bartley & Davies (1952).

This work was carried out on behalf of the Agricultural Research Council.

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The Hexokinase Activity of Rat Muscle Extract and the Lability of the Mg-ATP Complex. By C. Liébecq. (*Laboratoires de Biochimie, Université de Liège, Belgium*)

Optimal conditions for the measurement of the hexokinase activity of rat muscle extract have been briefly reported (Liébecq, 1952). A more careful analysis of the factors involved revealed that the requirements for adenosinetriphosphate and magnesium ions are linked; optimal activity occurs

when the Mg:ATP ratio equals 1. Excess ATP and, to a smaller extent, excess Mg^{2+} are inhibitory in a way which appears to be competitive. It is concluded that the actual phosphorylated substrate of the hexokinase reaction is the Mg-ATP complex rather than ATP itself. Similar but not identical observations had been made by Hers (1952) working with liver fructokinase.

The Mg-ATP complex is more unstable than ATP itself, as shown by measuring the spontaneous hydrolysis of ATP solutions at 30° in the absence and in the presence of magnesium ions. The catalysis by Mg^{2+} was more effective at pH 9·0 than at pH 7·0 and practically nil at pH 4·7. It was also shown that ATP may combine with one or two Mg^{2+} to form two complexes, the latter being the most unstable. Mg^{2+} is believed to withdraw the negative charges of the hydroxyl groups that protect the pyrophosphate bond against nucleophilic action as suggested by Lipmann (1951) and illustrated in experiments on acetyl-phosphate by Koshland (1951, 1952).

Optimal pH for the measurement of the hexokinase activity of rat muscle extract was found to be 7·8–7·9 and the pH-activity curve was found to be asymmetrical, the slope being steeper on the acid side where labilization of ATP by magnesium is lowest. The curve is believed to be the result of the effect of pH on both the electric configuration of the enzyme protein and the affinity of Mg^{2+} for ATP.

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Effect of Heparin and Heparin 'Clearing Factor' on Lipoid Transport in the Rabbit. By A. Comfort.* (*Department of Zoology, University College, W.C.* 1)

The injection of heparin, or the addition of plasma from heparin-injected rabbits, fails to reduce the turbidity of the plasma of rabbits on a high fat, high cholesterol diet (Anfinsen, Boyle & Brown, 1952). Injection of heparin does, however, produce changes in the ultracentrifuge pattern of rabbit blood lipoids (Graham, Lyon, Gofman, Jones, Yankley, Simonton & White, 1951). Similarly, although heparin added *in vitro* does not clear human plasma (Anfinsen *et al.* 1952), it affects the electrophoretic mobility of some components (Chargaff, Ziff & Moore, 1941; Nikkilä & Majanen, 1952; Nikkilä, 1952).

Littermate female rabbits of a mixed strain were fed on the lanolin-cottonseed oil diet of Dubach & Hill (1946). The pattern of cholesterol-bearing proteins in their plasma was studied by paper electrophoresis in barbiturate buffer and elution of the cholesterol (Comfort, unpublished), and lipoid bands were stained with Sudan Black B (Swahn, 1952). The animals were studied during the development of dietary hypercholesteraemia, and the plasma patterns before and after injection of heparin ('Pularin' Evans, 2000 units/kg.) compared.

In two out of five rabbits, only traces of an α-lipoprotein band were found, even at the height of the lipaemia, while in the remaining three an α-lipoprotein band appeared as soon as blood lipoids reached a level detectable by the methods used. These individual differences of pattern were consistent during the 16 weeks of the experiment.

Heparin added to lipaemic rabbit plasma (100 units/ml.) produced no significant change in turbidity, mobility of lipoid fractions, or mobility of stainable protein.

Heparin injected intravenously produced no reduction in the turbidity of plasma drawn after 60–90 min. It produced a marked increase in the anodic mobility of β-lipoproteins compared with pre-injection controls run on the same strip and a smaller increase in α-lipoprotein mobility.

Plasma of heparin-injected rats, active in clearing human lipaemic serum, produced no effect on the turbidity of rabbit plasma. Its effect on the electrophoretic pattern was like that of injected heparin but more marked.

The changes in anodic mobility produced by injected heparin were less evident, or even absent, at plasma cholesterol levels less than 0·5 mg./ml. It appears that in the rabbit heparin clearing factor, but not heparin *in vitro*, produces marked changes in the plasma lipoid pattern without significantly affecting the turbidity.

* Maintained by a grant of the Nuffield Foundation.

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The Inactivation of Human Red Blood Cell Tributyrinase. By HARRY O. MICHEL (introduced by D. R. DAVIES). (*Chemical Defence Experimental Establishment, Porton*)

Previous work done with radioactive DFP demonstrated that the radioactivity, due to ^{32}P, was stably bound to proteins in red blood cells and that not more than about 5% of the binding could be with the cholinesterase of the cells. In an attempt to account for the relatively large binding of DFP by the red cells the reactions of three anticholinesterases, DFP, TEPP and E. 600 (paraoxon) with the tributyrinase of human red blood cells was investigated. It was found that DFP rapidly inactivates red cell tributyrinase. A linear relationship was found between the degree of inactivation and the amount of DFP present in the solution containing tributyrinase. The amount of DFP necessary to produce inactivation of tributyrinase was found to be approximately equal to the amount of radioactive DFP which was bound by red cells containing the equivalent amount of tributyrinase. If red cells were saturated with tributyrin prior to the addition of radioactive DFP, virtually all binding of DFP was blocked. TEPP-inactivated red blood cell tributyrinase more slowly than DFP but the number of equivalents required for inactivation was the same. E. 600 reacted very slowly with red cell tributyrinase. The number of equivalents required for inactivation was somewhat greater than the value obtained with DFP. Due to the slowness of the reaction a linear relationship between degree of inactivation and amount of E. 600 present in the enzyme solution was not obtained. The reactions between tributyrinase and the inactivators used in this study were all bimolecular. Tributyrinase may account for virtually all the binding of such phosphorylating agents as DFP, TEPP and E. 600 by human red blood cells except for a relatively small fraction due to cholinesterase. The velocity constant for the breakdown of the enzyme-substrate complex of tributyrinase with tributyrin at pH 7·4 and 25° was estimated to be about 700 min.$^{-1}$.

Convulsions Induced by Fluorocitrate in Pigeons. (*Film.*) By R. A. PETERS and R. WAKELIN.

Héterogénéité de l'insuline cristallisée. By E. FREDERICQ.

Au sujet de la dégradation enzymatique des acides ribo- et desoxyribonucléiques. By L. VANDENDRIESSCHE.

Phénomènes d'agrégation dans les solutions d'hémoglobine. By P. PUTZEYS.

Trame protidique des nacres. By CH. GRÉGOIRE, GH. DUCHATEAU and M. FLORKIN.

Etude immunologique en gels d'agar et par électrophorèse de la fraction non infectieuse du virus de la mosaïque du tabac. By R. JEENER and G. LAVAND'HOMME.

La participation du cycle des acides tricarboxyliques au métabolisme azoté chez *Bacillus subtilis*. By J. WIAME, R. STORCK and S. BOURGEOIS.

Quelques données nouvelles sur le rôle du noyau cellulaire dans le métabolisme des protéines chez *Acetabularia mediterranea*. By H. CHANTRENNE, J. BRACHET and J. BRYGIER.

Etude chimique d'une cryoglobuline isolée. By J. P. Dustin and J. Leonis.

Amino-Acid Composition of an Electrophoretically Pure Sample of γ-Globulins. By R. Crokaert, H. Verbruggen and E. J. Bigwood.

The Action of Cysteamine on Liver Glycogen. By Z. M. Bacq and P. Fischer. (*La Laboratoire de Pathologie générale, Université de Liège*)

DEMONSTRATIONS

Determination of Amino-acids in Pure Proteins, in Foodstuffs and in Urine.

Various Models of Automatic Fraction Collectors.

Composition of Crystalline Papain, Bence-Jones Protein, γ-Globulins, Chorionic Gonadotrophin, Various Preparations of Hyaluronic Acid.

Amino-Acid Contents of Meat, Human Milk and Cow's Milk in Relation to Species and to Food Intake.

Amino-Aciduria in Infants; Histidinuria.

By E. J. Bigwood, J. P. Dustin, R. Crokaert, P. Soupart, E. Schram, E. L. Adriaens, J. Close, G. M. Ellinger, P. Van Geem, C. Czajkowska, C. Wodon and O. Medard.

La diazoréaction de la thyroxine et l'effet rénal de la 3′-(1-azo-2, 4-dichlorophényl)-3, 5, 5′-triiodothyronine. By G. Barac. (*Institut de Clinique et de Policlinique médicals, Hôpital de Bavière, Université de Liège*)

Historadiographie dans l'étude du développement embryonnaire. By A. Ficq. (*Laboratoire de Morphologie animale, Université libre de Bruxelles*)

Incorporation "in vitro" de glycine-1-C^{14} dans les cellules individuelles de la moëlle osseuse. By A. Ficq, F. Gavosto et M. Errera. (*Laboratoire de Morphologie animale, Université libre de Bruxelles*)

Le produit de la réaction de la fructokinase musculaire. By H. G. Hers. (*Laboratoire de Chimie physiologique, Université de Louvain*)

Action de la chloropicrine sur la ribonucléase. By L. Ledoux. (*Laboratoire de Morphologie animale, Université libre de Bruxelles*)

Mise en évidence de la dénaturation de l'acide désoxyribonucléique, par dialyse en présence de colorants basiques. By R. Thomas. (*Laboratoire de Morphologie animale, Université de Liège*)

[*The Editors of the Biochemical Journal accept no responsibility for the Reports of the Proceedings of the Society. Abstracts of papers read are published as received from the authors.*]

PROCEEDINGS OF THE BIOCHEMICAL SOCIETY

The **318th Meeting** *of the Biochemical Society was held in the Department of Physiology, The Medical School, Hospitals Centre, Birmingham* 15, *on Saturday,* 16 *May* 1953, *at* 1.45 *p.m., when the following papers were read:*

COMMUNICATIONS

The Metabolism of Pyruvate, Lactate and Glucose by Sheep-rumen Epithelium. By R. J. Pennington and T. M. Sutherland. (*Rowett Research Institute, Bucksburn, Aberdeenshire*)

It has been previously shown (Pennington, 1952) that the epithelial tissue of the fore-stomachs (rumen and reticulum) of the sheep produces relatively large amounts of ketone bodies from butyric and acetic acids, a property which they share with liver tissue.

Sections of rumen epithelial tissue readily metabolize pyruvate, aerobically, with the production of ketone bodies. During a 3-hr. incubation period, up to more than half of the pyruvate which disappeared, other than by reduction to lactate, was accounted for as ketone bodies. The proportion of ketone bodies formed was less in the presence of CO_2. When the metabolism of pyruvate (in presence of CO_2) was followed over successive periods it was found that the pyruvate which was metabolized other than to lactate after the first 1·5 hr. appeared practically quantitatively as ketone bodies. Possibly pyruvate does not readily undergo carboxylation, but some metabolite originally present in the tissue is carboxylated with the formation of a dicarboxylic acid. Respiration studies showed that the dicarboxylic acids of the Krebs cycle are metabolized by this tissue.

Ketone bodies were produced also from lactate, although the latter was metabolized much less rapidly than pyruvate.

In contrast, added glucose was metabolized without the production of ketone bodies. The endogenous ketone-body production was suppressed by glucose in the presence of CO_2. When glucose and pyruvate were metabolized together no ketone bodies were produced. The presence of glucose did not decrease the amount of pyruvate metabolized. Similar results were obtained with glucose + lactate.

The existence of a pathway for glucose metabolism, other than via pyruvate, is indicated, involving the formation of a dicarboxylic acid by a CO_2 fixation reaction.

In the presence of malonate (0·025 M) glucose uptake was not appreciably altered but was accompanied by the formation of ketone bodies.

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The Influence of Acetamide on the Citrate Accumulation after Fluoroacetate Poisoning. By S. Gitter (introduced by R. A. Peters). (*Department of Biochemistry, Oxford*)

It has been demonstrated (Gitter & Blank, 1952) that acetamide has a protective action in rats against fluoroacetate, when given simultaneously or from 30 min. to 24 hr. previously to fluoroacetate. The 88 % mortality rate, obtained by subcutaneous injection of 10 mg./kg. of ethylfluoroacetate, was lowered to 33 % by acetamide. Other protective features were: prolongation of the latent period; shorter and less frequent convulsions; longer period of survival, even of animals that die in the end.

No protection was obtained when the dose of fluoroacetate was much increased, nor when acetamide was given during the latent period before convulsions had started, 30 min. after fluoroacetate.

The protective results obtained induced us to believe that acetamide might belong to those —C—C substances which, as postulated by Peters (1952), penetrate into the mitochondria and thereby get a chance to interfere with the formation of the fluorotricarboxylic acid.

An intraperitoneal dose of 5 and 10 mg./kg. of sodium fluoroacetate was given to male white rats, and citrate estimations in brain, heart and kidney homogenates were carried out after 30 and 60 min. Half of the animals had received a subcutaneous dose of 1·25 g./kg. of acetamide 2 hr. before the fluoroacetate injection.

Acetamide reduced the citrate in heart and kidney, but not in brain, as may be seen from the citrate ratio, i.e. percentage of (citrate formed in presence of acetamide)/(citrate formed without acetamide).

It is assumed that acetamide interferes with the condensation of fluoroacetate to fluorocitrate. The total amount of fluorocitrate produced is therefore reduced, causing a less pronounced inhibition of aconitase (cf. Lotspeich, Peters & Wilson, 1951) and correspondingly lower citrate values. Still, some fluorocitrate is formed, even in the presence of acetamide, and this accounts for the failure of acetamide to protect when too much fluoroacetate is given. It is now also understood why acetamide cannot protect 30 min. after fluoroacetate, as by then a toxic concentration of fluorocitrate would be already present within the cells. Acetamide proved, however, to produce a valuable protection when given 8 min. after fluoroacetate, when apparently not enough fluorocitrate has yet been produced and further condensation of fluoroacetate into fluorocitrate is still efficiently inhibited by acetamide.

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The Catalytic Effect of Molybdate on the Hydrolysis of Organic Phosphates. By H. Weil-Malherbe. (*Research Department, Runwell Hospital, Wickford, Essex*)

In a recent paper Lutwak & Sacks (1953) described experiments in which organic phosphates were heated at 100° in solutions containing N-acid and 1 % ammonium molybdate. They found that results were higher when phosphate was estimated colorimetrically in the solution than when it was first precipitated. They also found with the direct colorimetric method a colour development indicating a hydrolysis in excess of 100 %, and in one case in excess of 400 %. These observations were interpreted by them as showing the formation of organic phosphomolybdate complexes.

It will be shown that the effect found by Lutwak and Sacks is observed only if a blue colour develops during the exposure to heat. The effect is similar whether the solution contains, e.g., fructose phosphate or a mixture of fructose and phosphate, and is therefore unlikely to be due to the formation of organic phosphomolybdate complexes.

It is suggested that the effect is due to three factors:

(1) If magnesia mixture is added to the *reduced* phosphomolybdate complex and the resulting precipitate analysed colorimetrically, recoveries of inorganic phosphate are low.

(2) There is a reduction of free molybdic acid resulting in the formation of molybdenum blue. Contrary to prevailing opinions, molybdenum blue is extracted from acid solutions by *iso*butanol. Solubility of the blue colour in *iso*butanol is therefore no reliable criterion for the absence of molybdenum blue.

(3) The specific extinction coefficient is about 4–5 times higher when the colour of the reduced phosphomolybdate complex is developed at 100° than when it is developed at room temperature. This is true for most reducing agents, with the exception of stannous chloride, with which a high extinction is already obtained at room temperature.

The effect of Lutwak and Sacks is not observed under the milder experimental conditions which were employed in the investigations of Weil-Malherbe & Green (1952). If it had occurred, a stannous chloride method of phosphate estimation, such as was used by these authors, would have been less subject to error than the method of Fiske and Subbarow used by Lutwak and Sacks.

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The Use of Specific Inhibitors in the Estimation of the Pseudo Cholinesterase in Nervous Tissue. By BRENDA J. BAYLISS and A. TODRICK. (*Department of Experimental Psychiatry, The Medical School, Birmingham* 15)

Ord & Thompson (1952) have drawn attention to the inadequacy of the existing method of estimating pseudo cholinesterase in nervous tissue. The rate of hydrolysis of butyryl and benzoyl choline by acetyl cholinesterase is appreciable (4·5 and 1·5 % of the rate of hydrolysis of acetyl-β-methylcholine respectively (Adams, 1949), and, since the concentration of this enzyme in nervous tissue is high, it may account for up to half of the activity usually attributed to pseudo cholinesterase. An attempt to improve the method by employing a specific inhibitor of acetyl cholinesterase has therefore been made.

A number of inhibitors of acetyl cholinesterase have been examined for their capacity to give adequate inhibition at a concentration which would have little or no effect on the pseudo cholinesterase. Some specific inhibitors of pseudo cholinesterase were also examined. Wellcome 62C47 (Glock & Mogey, 1948) and Wellcome 284C51 were found to inhibit 97–98 % of the acetyl-cholinesterase activity at concentrations (of 10^{-5} and 3×10^{-5} M respectively) which only inhibited pseudo cholinesterase by 1–2 %. Ethopropazine methosulphate, 'Lysivane', a specific inhibitor of pseudo cholinesterase, was less effective; at a concentration of 5×10^{-5} M it inhibited the acetyl and pseudo cholinesterases by 5 and 95 % respectively.

Measurements of the hydrolysis of benzoyl-choline by rat-brain homogenate in the presence and absence of Wellcome 62C47 and 'Lysivane' have been made. The sum of the activities determined in the presence of the inhibitors (which were used singly) is equal to the total activity in the absence of inhibitors, after allowing for substrate and tissue blanks.

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The Development of the Cholinesterases in the Brain and Spinal Cord of the Young Rat. By BRENDA J. BAYLISS and A. TODRICK. (*Department of Experimental Psychiatry, The Medical School, Birmingham* 15)

The acetyl and pseudo cholinesterase levels have been estimated in rat brain and spinal cord between the third and thirtieth days after birth.

From the third to the twentieth day the acetyl-cholinesterase activity of the whole brain increases at slightly more than 300 units (μl./hr.) per day; there is a fall in rate after this age.

The pseudo cholinesterase activity per unit weight remains constant at 150–160 units between the third and tenth days; this is followed by an increase which continues up to about the twenty-fourth day, when it reaches a level of 300 units.

Acetyl and pseudo cholinesterase activity in spinal cord homogenates show no significant change per unit weight between the eighth and twenty-second days after birth. Both enzymes are initially present in higher concentrations than in the brain.

According to Craigie (1938), capillaries increase rapidly in the rat brain, and the differences which occur in the adult become established between the tenth and twenty-first day. Koelle (1952), on the basis of histochemical evidence, has suggested that the pseudo cholinesterase of the rat brain is principally located in the walls of the capillaries, the muscle fibres of the arterioles and venules, and some glia (especially the fibrous astrocytes). These two findings may not be unrelated to the rise in pseudo cholinesterase activity between the tenth and twenty-fourth day reported above.

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The Variation with Age of the Hydrolysis of Choline Esters by Rat Tissues. By J. E. Risley and D. R. Davies. (*Chemical Defence Experimental Establishment, Porton, Wilts.*)

The variation with age of the ability of rat tissues to hydrolyse a series of choline esters has been studied.

Against acetylcholine the hydrolytic activity of brain tissues increases from birth to four times the activity when fully grown. Against acetyl-β-methylcholine (MCh), benzoylcholine (BCh), butyrylcholine (BuCh) and propionylcholine (PCh) similar changes are seen. Spinal-cord activity increases from birth to a maximal value over the ensuing 4 weeks. Afterwards a steady decrease occurs, and the adult level is only half the level at birth and one-third the maximum level observed. This pattern is essentially true for the other substrates, although some slight variations in detail occur.

Skeletal muscle obtained from the leg shows a maximal level within 7 days of birth. Then a slight fall in activity occurs. The level remains comparatively constant for the next month, and thereafter a marked fall occurs and the level in the adult rat is only 10% or even less than that observed during the first week of life. The relative MCh activity at birth is only a quarter of the ACh activity. The proportion increases with age, and in the adult animal MCh activity is three-quarters the ACh. With BCh and BuCh, on the other hand, the relative activity falls steadily until in the adult the activity of the muscle towards these substrates is negligible. The ratio of PCh activity to ACh activity changes markedly with age. Thus, during the first week of life rat muscle hydrolyses PCh very much faster than ACh. In succeeding weeks, however, the ratio decreases until in the adult the PCh activity is less than the ACh. Heart-ventricle activity changes relatively little throughout life. A steady decrease occurs from birth to about half this value in maturity. This is true with the other four substrates; but at all periods PCh activity is about twice that of ACh.

Relatively little data have been obtained from the liver and kidney. Liver appears to increase during the first 3 weeks of life. The adult level is, however, the same as at birth. MCh activity appears to decrease relative to ACh. Both BuCh and PCh are hydrolysed faster than ACh. In the case of the kidney the ACh activity falls off with increasing age. Its final value with all substrates is less than one-tenth the value at birth. BuCh and PCh are again hydrolysed much faster than ACh.

These results are discussed.

The Purine and Pyrimidine Content of Some Deoxynucleic Acids from Tumours. By D. L. Woodhouse. (*Cancer Research Laboratories, The Medical School, University of Birmingham*)

Deoxynucleic acids have been prepared and purified by modifications of Mirsky's method, using, amongst others, the following tumours as source material: transplanted mouse and rat sarcomata, transplanted mouse-breast tumours from C_3H and IF mice, spontaneous breast tumours from C_3H mice, transplanted rat Walker tumour, rat-liver tumour induced by 2-acetamidofluorene, human carcinoma of breast and carcinoma of uterus. These have been analysed for purine and pyrimidine content by standard methods of hydrolysis, chromatography, and ultraviolet-absorption measurements.

The contents and ratios are essentially of the type with adenine/guanine, and thymine/cytosine > 1. While certain differences in content are apparent compared with specimens from normal tissues, these do not suggest any constant feature distinguishing the DNA of tumour cells.

The Effect of Nucleic Acids and other Compounds on the Coagulation of Serum Albumin by Heat. By D. Hamer. (*Cancer Research Laboratories, The Medical School, University of Birmingham*)

It has been reported by other workers that comparatively small amounts of thymus nucleic acid could prevent the coagulation of dialysed albumin solutions by heat (Greenstein & Hoyer, 1950). It was suggested, however, that this effect was characteristic of thymus nucleic acid and depended on its particular structure.

In the present work, while the above experimental observations have been confirmed, the interpretation proposed needed extending, since it has been found that other acidic compounds of high molecular weight have a similar protective effect. For example, 0·003% dextran sulphate in 0·75% albumin solution prevents coagulation completely.

Dextran of similar molecular weight has no effect, showing that the presence of acidic groups is essential to the protective effect. A number of other high-molecular-weight polyacids have been studied in which the functional acid groups were phosphate, sulphate and carboxylate, and all were found to have a similar effect. Details of these experiments will be presented and the mechanism of the protective action discussed.

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An Amidase of Rabbit Liver. By H. G. Bray, K. White and P. B. Wood. (*Department of Physiology, University of Birmingham*)

Earlier work with this enzyme (Bray, James, Ryman & Thorpe, 1948; Bray, James, Raffan, Ryman & Thorpe, 1949; Bray, James, Thorpe, Wasdell & Wood, 1949; Bray, James, Thorpe & Wasdell, 1950) was concerned mainly with a comparison of the results of its action *in vitro* with those obtained for the hydrolysis of amides in the intact rabbit. The mechanism of the enzymic process is now being studied. For this purpose *n*-hexanamide has been chosen as substrate for preliminary investigation. It has been shown that the action of the amidase is inhibited by excess of substrate and by *n*-hexanoate ions; ammonium ions have no significant effect. Certain other carboxylic acids inhibit the hydrolysis of *n*-hexanamide. The effects of variations in temperature, pH and solvent composition have also been studied. Some preliminary results will be presented and their mathematical treatment discussed.

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The Metabolism of 2:3:4:6-Tetrachloronitrobenzene and Pentachloronitrobenzene in the Rabbit. By J. J. Betts, Sybil P. James and W. V. Thorpe. (*Department of Physiology, University of Birmingham*)

2:3:4:6-Tetrachloroaniline and *N*-acetyl-*S*-(2:3:4:6-tetrachlorophenyl)-L-cysteine have been isolated from the urine of rabbits dosed with 2:3:4:6-tetrachloronitrobenzene. An increased excretion of glucuronide was also observed corresponding in extent to the hydroxylation of about 37 % of the dose. The extent of ethereal sulphate formation was very small. Only about 53 % of the 2:3:4:6-tetrachloronitrobenzene fed has been accounted for. No unabsorbed compound was detected in faeces.

The only metabolites isolated after giving pentachloronitrobenzene were pentachloroaniline and *N*-acetyl-*S*-(pentachlorophenyl)-L-cysteine. These metabolites account for about 19 % of the dose. Only half of the dose was absorbed.

The biosynthesis of the mercapturic acids from 2:3:4:6-tetrachloronitrobenzene and pentachloronitrobenzene affords two further examples of the attachment of an acetylcysteyl group to a carbon atom of the benzene ring with the concomitant elimination of a nitro group. This was first observed by Bray, Hybs, James & Thorpe (1952) with 2:3:5:6-tetrachloronitrobenzene. In the case of pentachloronitrobenzene, the acetylcysteyl group must enter the position originally occupied by the nitro group.

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The Kinetics of Ethereal Sulphate Formation in Tissue Slices. By H. G. BRAY, A. HODGKINSON and K. WHITE. (*Department of Physiology, University of Birmingham*)

The kinetics of ethereal sulphate formation have been studied *in vivo* (Bray, Humphris, Thorpe, White & Wood, 1952) and also in tissue slices and suspensions (de Meio & Tkacz, 1952; Bernstein & McGilvery, 1952). Tissue-slice preparations permit more extensive variations in the concentrations of reactants than are possible in the intact animal. In most of the present experiments 3:5-xylen-1-ol has been used as the phenol, since it forms an ether-soluble glucuronide which facilitates study of the main concomitant reaction, glucuronide formation. By following changes in the phenol and sulphate-ion concentrations in medium and rabbit-liver slices it has been concluded that both substances enter the cell by passive transfer. The permeability of slices to sulphate ions appears to decrease with time. Pre-incubation of liver slices with sulphate ions, but no phenol, increases the extent of ethereal sulphate formation by the slices when the phenol is added. Some preliminary experiments on the relationship between the concentrations of reactants and the rate of ethereal sulphate formation will be discussed.

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The Enzymic Decomposition of Blood-group *H* Substance derived from Pig Mucin. By M. J. CRUMPTON and W. T. J. MORGAN. (*Lister Institute, London, S.W.* 1)

Enzymes able to destroy the serological specificities of the human and animal blood-group substances (*A*, *B*, *H* and Lewis Le^a) are known. Culture filtrates from *Clostridium welchii* (type *B*) have been most frequently used, the enzyme being purified by precipitation from the filtrate by acetone at $-10°$ and repeated ammonium sulphate fractionation from McIlvain buffer (pH 6·8; phosphate 0·05M) at 0° between the levels 25–31%, w/v, saturated ammonium sulphate. The purified enzyme had 321 *H* units/mg. N; 268 *A* units/mg. N; N, 9·3% and a molecular weight of about 70000. Material with a greater *A* enzyme activity, relative to *H* activity, was obtained above 31% saturation but complete separation of the two enzymes was not achieved by this means. *H* substance (903 mg. N, 6·1%; fucose, 10·6%; reducing sugar, as glucose, and hexosamine as glucosamine after hydrolysis, 51 and 31·6% respectively) in buffer of pH 6·8 was incubated for 5 hr. at 37° in the presence of toluene with an amount of unheated enzyme which contained 2·9 mg. N. The substrate could be recovered unchanged after incubation with heat-inactivated enzyme.

The enzymically degraded substrate was separated by dialysis into diffusible and indiffusible components. The latter consisted of 210 mg. of a water-insoluble material (N, 8·65%; fucose, 3·7%; reducing sugars, 38·1%; and hexosamine, 21·5%) and a water-soluble component (298 mg. N, 7·6%; fucose, 3·8%; reducing sugars, 45·1%; hexosamine, 26·3%). Both materials appeared to be homogeneous in the ultracentrifuge. The diffusate contained 16·4 mg. N which was accounted for by diffusible enzyme and 223 mg. of *N*-acetylglucosamine; fucose (84 mg.) and galactose (67 mg.) were also present. Ratio of the sugars 8:4:3 respectively. An *N*-acetyl-chondrosamine-galactoside was identified chromatographically.

The *H* activity of the indiffusible material was unchanged when measured with a human anti-*H* serum (Warboys) but was less than 1% of its original value when tested with a rabbit anti-*H* immune serum. None of the enzymic hydrolysis products precipitated with pneumococcus type XIV horse serum.

Enzyme previously heated to 55° for 10 min. brought about less extensive degradation of *H* substance, but the same sugars, in the ratio 11:8:10, were nevertheless detected in the diffusate.

The Serological Inactivation of the Human Blood-group Substances by an Enzyme Preparation obtained from *Trichomonas foetus*. By WINIFRED M. WATKINS. (*Lister Institute of Preventive Medicine, London, S.W.* 1)

The enzyme systems of Protozoa have not been extensively investigated largely owing to the difficulties encountered in the past in growing the organisms in pure culture on a large scale. The development of media upon which the Protozoa can grow and divide normally and, in certain instances, the use of antibiotics to isolate pure strains of Protozoa have now enabled several species to be grown free from bacteria in sufficient quantities for enzyme studies.

The action on the human blood-group substances of three Protozoa, *Trichomonas foetus*, *Strigomonas oncopelti* and *Tetrahymena pyriformis*, kindly supplied by Dr Muriel Robertson, F.R.S., and of a fourth, *Trichomonas vaginalis*, kindly supplied by Dr J. G. Feinberg, has been examined. Cell-free extracts of the organisms were prepared by autolysis of living cultures followed by centrifugation. Extracts of *T. foetus*, of both the Belfast and Manley strains, contain enzymes which rapidly destroy the serological activity of the water-soluble *A*, *B*, *H*, Le^a and Le^b substances. Similarly prepared extracts of the other organisms are without action on the blood-group mucoids. The extracts of *T. foetus* do not destroy the serological properties of the specific polysaccharide from *Shigella Shigae*, the type XIV pneumococcus polysaccharide, or the specific substance isolated from *T. foetus* (Feinberg & Morgan, 1953).

Partial purification of the *T. foetus* extract by ammonium sulphate fractionation gives a product with increased activity against human *H* substance, but the preparation no longer inactivates the *A*, *B* or Lewis substances. Preliminary examination of the properties of the *H*-destroying enzyme in the purified product suggests that the enzyme has a pH optimum between pH 6 and 7, and a temperature optimum at about 45°; the activity of the preparation is lost completely on heating at 56° for 30 min. In certain respects the enzymes present in the *T. foetus* preparations appear to differ from the blood-group-destroying enzymes obtained from culture filtrates of *Clostridium welchii* (Stack & Morgan, 1949).

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The Structure of 'Insoluble' Laminarin. By STANLEY PEAT, W. J. WHELAN and H. G. LAWLEY. (*Department of Chemistry, University College of North Wales, Bangor*)

The work of Barry (1939) and of Connell, Hirst & Percival (1950) showed that the chain-forming linkage between the glucose units of the seaweed polysaccharide, laminarin, is of the β-1:3 type, but left unresolved the question of the possible existence of branch linkages. The experiments now reported indicate the presence of such linkages.

'Insoluble' laminarin (21·2 g.) from *Laminaria cloustoni* was partly hydrolysed by being heated with 0·33 N-sulphuric acid for 2 hr. and the neutralized solution fractionated on charcoal-Celite (Whistler & Durso, 1950). The monosaccharide fraction contained glucose and a small amount of fructose; the disaccharide fraction contained three members, identified by the physical properties of the sugars and of their crystalline β-octa-acetates as laminaribiose (2·5 g.), gentiobiose (0·1 g.) and $\beta\beta$-trehalose (0·1 g.). Five trisaccharides were obtained, one being crystalline and non-reducing. Paper chromatographic examination of the products of partial acid hydrolysis of each trisaccharide revealed that the non-reducing sugar yielded $\beta\beta$-trehalose and laminaribiose, three of the trisaccharides yielded laminaribiose and gentiobiose, while the fifth member of the group (obtained in largest amount) yielded only laminaribiose and was therefore presumed to be laminaritriose.

We have evidence that the di- and tri-saccharides are not acid reversion products, and it would appear therefore that this laminarin, hitherto believed to possess a linear structure, is in fact branched. The chains are formed by β-1:3-links between glucose members and the branch points by β-1:6-linkages. The $\beta\beta$-trehalose could arise from a point of union through the reducing groups of two such branched molecules or from a β-glucose unit combined with the reducing end of one such molecule. These structures would yield, on acid hydrolysis, laminaritriose, three trisaccharides each containing a β-1:3- and a β-1:6-linkage, and a non-reducing trisaccharide containing the $\beta\beta$- and β-1:3-linkages. Further proof of the supposed nature of these tri-

saccharides has resulted from an examination of the products of partial hydrolysis by β-glucosidase (almond emulsin).

It may well be that synthesis of laminarin in the plant proceeds by enzymic transglucosylation from a ββ-trehalose substrate. This process is known to occur in the synthesis of certain polysaccharides from sucrose or maltose. This possibility is now being examined.

We are indebted to Dr E. T. Dewar for the gift of 'insoluble' laminarin.

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β-Methyl Fructoside as a Substrate in Transfructosylation. By W. J. Whelan and D. M. Jones.
(*Department of Chemistry, University College of North Wales, Bangor*)

The study of transfructosylation reactions of yeast invertase is facilitated by the use of β-methyl fructoside rather than sucrose as substrate, since the separation of synthetic disaccharides from unchanged sucrose is thereby avoided. β-Methyl fructoside is prepared by the reaction of sucrose and methanol in aqueous solution in presence of yeast invertase (British Drug Houses) (Bacon, 1952). Transfructosylation has been shown to occur with simple primary alcohols (ethyl, propyl, butyl; see also Bacon, 1952) but not with secondary or tertiary alcohols (propyl, butyl). Monosaccharides containing a primary hydroxyl group (glucose, mannose, galactose) act as 'acceptors' for fructose units transferred by invertase from β-methyl fructoside (evidence of paper chromatography). Sorbitol and mannitol function particularly well as acceptors, but sugars from which primary hydroxyl groups are absent (arabinose, ribose, rhamnose) fail to react.

The product of transfer to D-glucose was isolated in yields of approx. 100 mg. from digests containing β-methyl fructoside (2·5 g.), glucose (12·5 g.), 0·2 M-acetate buffer (pH 4·8; 2·5 ml.) and invertase (2·5 ml.) incubated for 15 min. at room temperature (19°). Fractionation was accomplished on charcoal-Celite (Whistler & Durso, 1950). The product ($[\alpha]_D + 5{\cdot}5°$ in water) was copper-reducing and was converted by invertase into glucose and fructose. Its reduction with sodium borohydride gave a non-reducing product ($[\alpha]_D - 23{\cdot}8°$ in water) which invertase converted into fructose and sorbitol. Oxidation with sodium metaperiodate liberated 3·89 moles of formic acid per mole of disaccharide. On this evidence and on the assumption that invertase is specific for the β-fructofuranosyl group, the synthetic disaccharide is accordingly characterized as 6-[β-D-fructofuranosyl]D-glucose (theoretically yielding 4 moles of formic acid). Two other products of this reaction were also detected in small amount. They are thought to arise by transfer of fructose units to β-methyl fructoside and free fructose respectively.

It would appear that this fructosyl glucose is the same as the hitherto unidentified product ('Spot 1') of the action of yeast invertase on sucrose reported by Bacon & Edelman (1951) and the disaccharide containing fructose and glucose isolated by White & Secor (1952). It is of interest to note that in the two known types of fructosan the chain linkage involves in each case one of the primary hydroxyl groups of fructofuranose, that at C 1 in inulin and at C 6 in the levans. These same hydroxyl groups are also involved respectively in the enzymic synthesis from sucrose of the trisaccharides, inulobiosyl-D-glucose (Pazur, 1952) and kestose (Albon, Bell, Blanchard, Gross & Rundell, 1953).

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The Biosynthesis of Fat and Cholesterol *in vitro* by Ovarian Tissues of the Laying Hen. By G. Popják and A. Tietz. (*National Institute for Medical Research, Mill Hill, London, N.W. 7*)

It has been observed some time ago in this laboratory that 5 or 6 days after the injection of [^{14}C]acetate into laying hens exceptionally large amounts of ^{14}C appeared in the lipids of the eggs, and that the hens continued to lay labelled eggs for several weeks. This observation suggested that the ovarian tissues themselves might be synthesizing and laying down yolk lipids. It was found that the membranes surrounding the growing ovum and which consist of the theca interna and granulosa synthesize *in vitro* fatty acids and cholesterol from [^{14}C]acetate. The yolk (deutoplasm) and the germinal disk have no synthetic ability under the conditions tested, neither do they influence in any way the synthetic activity of the membranes. Addition of glucose (0·3 %) or of oxaloacetate (0·02 M), and to a lesser extent of α-ketoglutarate, caused stimulation of fatty-acid synthesis; pyruvate and succinate were without effect. While glucose stimulated acetate oxidation, oxaloacetate caused a marked inhibition. Although these membranes are inferior to liver slices of young rats in respect of fatty-acid synthesis, they are as active as liver slices (on a wet-weight basis) in synthesizing cholesterol from acetate. Pyruvate, oxaloacetate and succinate caused some inhibition of cholesterol synthesis, while glucose and α-ketoglutarate had no effect. The younger the ova the more active are the membranes surrounding them in oxidizing acetate, and in synthesizing fatty acids and cholesterol, although even the old membranes undergoing involution after ovulation are still highly active.

Although these membranes are not very highly cellular they showed a fairly high endogenous $-Q_{O_2}$ with a mean of $2{\cdot}92 \pm 0{\cdot}26$ (10 determinations). Further, they appear to be rich in oxidizable substrates as they continue to respire for at least 18 hr. with only a slight change in $-Q_{O_2}$ in the absence of added substrate. Acetate caused regularly a slight rise (5–10 %) in $-Q_{O_2}$; glucose (0·3 %) had a similar effect only after the membranes were allowed to respire for several hours without added substrate. Oxaloacetate, α-ketoglutarate and succinate (0·02 M) caused a 25–30 % increase, while pyruvate (0·02 M) caused a similar decrease in $-Q_{O_2}$.

Fatty-acid Synthesis in Slices and Homogenates of Rat Mammary Gland. By G. Popják and A. Tietz. (*National Institute for Medical Research, Mill Hill, London, N.W. 7*)

Mammary-gland slices of lactating rats do not synthesize fat from acetate when acetate is the only substrate in the incubation medium; in the presence of glucose, however, acetate is used for fatty-acid synthesis (Folley & French, 1950). We find that substrates other than glucose, e.g. pyruvate, oxaloacetate, α-ketoglutarate or succinate, also stimulate fatty-acid synthesis from acetate, although not to the same extent as glucose. Oxaloacetate had the greatest effect next to glucose on fatty-acid synthesis, and in addition it inhibited oxidation of acetate, while all other substrates either stimulated, or had no effect on, acetate oxidation. Homogenates, prepared from rat mammary glands in a tightly fitting glass homogenizer and freed from large cell debris by centrifugation at 400 *g* for 10 min., synthesize fatty acids from [^{14}C]acetate after suitable additions. Homogenates, like slices, do not synthesize fatty acids when acetate (0·02 M) or acetate (0·02 M) + ATP (0·01 M) are the only substrates added to the medium. Glucose (0·3 %), either alone or in combination with ATP, does not promote fatty-acid synthesis from acetate by homogenates. The highest incorporation of [*carboxy*-^{14}C] acetate into fatty acids by these homogenates has so far been observed in the presence of acetate, 0·02 M, oxaloacetate, 0·02 M, and ATP, 0·01 M. Neither oxaloacetate nor ATP alone caused a significant stimulation of fatty-acid synthesis from acetate by homogenates. Changing the gas phase in the flasks from pure O_2 to air caused a nearly three-fold increase in the amount of acetate incorporated into fatty acids in the presence of oxaloacetate + ATP; the $-Q_{O_2}$ also increased from about 1·6 to 2·2–2·4. Incubation under N_2 caused a 70–90 % inhibition of fatty-acid synthesis. α-Ketoglutarate (0·02 M), in contrast to oxaloacetate, stimulated fatty-acid synthesis even without added ATP; ATP added to α-ketoglutarate caused a further stimulation of fatty-acid synthesis from acetate.

The fatty acids synthesized from [*carboxy*-^{14}C] acetate by four homogenates in the presence of oxaloacetate + ATP were pooled and resolved into individual acids by chromatography. All saturated *n*-fatty acids from C_6 to C_{18} and also oleic acid were obtained. The specific activities (counts/min./C in infinite thickness) of the saturated acids were: C_6, 6060; C_8, 603; C_{10}, 325; C_{12}, 657; C_{14}, 925; C_{16}, 960; C_{18}, 108; the specific activity of oleic acid was 382.

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The Actions of α-Amylases. By R. H. HOPKINS and R. BIRD. (*Department of Applied Biochemistry, University of Birmingham*)

In order to study the mode of attack by the α-amylases in their initial stages, very dilute reaction mixtures were used. This minimizes hydrolysis of low molecular fission products (Somogyi, 1940). Following the reactions by paper chromatograms (Partridge, 1948, 1949) using mixtures of glucose, maltose, maltotriose, etc., for controls, it was found that when only 10–15 % of the linkages in amylose (0·025 %) had been split by salivary amylase there were already much maltose and maltotriose present, suggesting that these sugars can be readily split from long chains. This is in agreement with the conclusion of Roberts & Whelan (1951) that fission is random except for the terminal linkages. When, however, amylopectin (0·027 %) was hydrolysed, maltose was not observed in the early fission products, whereas maltotriose and higher saccharides appeared in relatively considerable quantities.

Amylose, compared with amylopectin of same concentration, contains relatively few non-reducing chain ends. But at a suitable stage of α-amylolysis it would contain as many as unhydrolysed amylopectin, and in addition a large number of reducing ends, in this respect differing from amylopectin. The appearance of maltose in amylose fission products is attributed to an attack on the second linkage from the reducing end, its absence from amylopectin fission products to the absence of reducing ends in the initial stage. The second linkage from the non-reducing end is not attacked. Thus one linkage at the reducing end and two at the non-reducing end are not subject to random fission.

In parallel experiments with the α-amylases of malt (crystalline) and *B. subtilis* products of lower chain length than six could not be detected in early amylopectin fission products, but were present when amylose was used. It is concluded that these amylases can, only with difficulty, attack the first five linkages from the non-reducing end of a chain. In confirmation, a linear dextrin of chain length 20 yielded maltose, maltotriose, tetraose and longer fission products in the early stages of salivary amylolysis while the iodine stain still indicated the presence of fragments of over eight units. α-Malt amylase and bacterial amylase yielded only units of 6–7 chain length in the corresponding stages.

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Bromsulphalein Retention in Choline Deficiency and Replacement in the Rat. By N. D. AHMAD and A. C. FRAZER. (*Department of Pharmacology, University of Birmingham*)

Thirty male rats of the Wistar strain weighing between 80 and 110 g. were put on the choline-deficient diet prescribed by Ridout, Lucas, Patterson & Best (1952). Fifteen rats of the same weight range were used as 'control' and were given choline-containing diet. Occasionally a few animals were killed and their livers stained with Sudan III and examined for the appearance of fat. After 4 weeks the livers of animals on choline-free diet were found loaded with fat. Fourteen of these animals were then subjected individually to Bromsulphalein (BSP) Liver Function Test (Ahmad & Frazer, 1952) and the BSP retention percentage determined for each animal. The average BSP retention in this group was 10·5 %, the highest being 17·2 %, whereas in the 'control' animals the average BSP retention was 3·6 %. Four animals from this group of fourteen were selected at random and killed. Their livers were sectioned and stained for fat and the extent of fatty infiltration noted.

The remaining ten animals were kept on the same diet which they were previously getting, but to which 0·12 % choline chloride had been added. After feeding these animals on choline-containing diet for a fortnight, their liver functions were estimated again. In all the animals except two the liver function, as estimated by BSP retention test, had returned to normal, All the animals, except the two which had shown abnormal BSP retention, were then killed and their livers examined histologically. These were completely free from fat and showed no other evidence of abnormality. The two animals which were spared and had shown abnormal retention were kept on choline-containing diet for another fortnight and their liver function estimated once again. As the BSP retention value was still well above normal, it was presumed that the fatty infiltration of the parenchyma had failed to clear up. Therefore the choline-containing diet was continued for another fornight. The liver function was

again estimated, and, as it was still abnormal, the animals were killed and their livers examined carefully to detect the lesion responsible for this persistent hepatic dysfunction in spite of feeding a balanced diet for over 6 weeks. Histopathological examination revealed no fatty infiltration, or any other evidence to account for high BSP retention, even after the dietary-induced fatty infiltration had cleared up.

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Studies on the Effects of Chlorine Dioxide on Gluten. By A. C. Frazer, P. Meredith and H. G. Sammons. (*Department of Pharmacology, University of Birmingham*)

Since Bentley, McDermott, Moran, Pace & Whitehead (1950) showed that methionine sulphoximine was produced from zein by the action of nitrogen trichloride, attention has been directed towards possible substitutes for it. Recent work by Moran, Pace & McDermott (1953) has indicated that the action of chlorine dioxide (ClO_2) on gluten does not produce any unusual amino-acids unless the treatment is much greater than normal. Chlorinated tyrosines were produced only when free chlorine was present in the ClO_2.

We have studied the amino-acid content of the gluten in untreated flour, flour treated with the normal amount of ClO_2, and flour treated with ten times the normal amount. The separated glutens were hydrolysed with 6N-HCl; 6N-HCl in N_2 and 5N-$Ba(OH)_2$. After hydrolysis the amino-acid patterns were studied by two-dimensional paper chromatography, using phenol-butanol-acetic acid. No differences in the amino-acid pattern of the three flours could be detected. No methionine sulphone or cysteic acid was found.

An unknown ninhydrin reacting, but not reducing, substance has been detected in all neutralized hydrolysates when kept at room temperature for 3 days, or for 5 days at 0°, but not when kept in the presence of chloroform at 0°. It is considered to be an artifact produced by bacterial contamination. It lies between histidine and methionine sulphone on the chromatogram.

During one acid hydrolysis samples were taken at intervals, starting at $\frac{1}{4}$ hr., then $\frac{1}{2}$ hr., and so on up to 36 hr. They were all examined chromatographically. Free tryptophan was detected in samples at $\frac{1}{4}$ hr., but not subsequently.

It is concluded that chlorine dioxide has not been shown to produce unusual amino-acids in wheat-flour protein within the limits of the methods used even when the flour is treated with ten times the normal amount. Nevertheless, the gluten is modified by ClO_2. With ten times the concentration used commercially it loses its coherence and becomes crumbly. There is also a colour change. These changes render it useless for baking. It is intended to publish full details of this investigation shortly.

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The Intestinal Absorption of Liquid Paraffin in the Rat. By J. W. Daniel, A. C. Frazer, J. M. French and H. G. Sammons. (*Department of Pharmacology University of Birmingham*)

Liquid paraffin is not absorbed when fed as such to rats. When the hydrocarbon is emulsified and introduced into an intestinal loop absorption of the oil can be demonstrated, provided that the particle size of the emulsion is of the order of 0·5 μ. (Frazer, Schulman & Stewart, 1944; Shoshkes, Geyer & Stare, 1950). When a 50 % (v/v) solution of liquid paraffin in olive oil was fed to rats for a period of 3 hr., 60 ± 5 % of the liquid paraffin was absorbed. This was confirmed by long-term studies. 170 g. of liquid paraffin dissolved in 177 g. olive oil were fed to ten rats over a period of 16 days, mixed with the normal diet. 62 g. of the mineral oil were recovered from the faeces, indicating that 65 % of the amount ingested had been absorbed. When 140 g. of the liquid paraffin alone were fed in the diet to five rats

for 28 days, 128 g. were recovered from the faeces (Daniel, Frazer, French & Sammons, 1951).

After feeding a solution of the hydrocarbon in olive oil, liquid paraffin was isolated from the unsaponifiable fraction of the lipids of the intestinal mucosa. Analysis of this material gave C, 86·2 and H, 13·8. The original liquid paraffin fed showed C, 86·0 and H, 13·8. From the amount of liquid paraffin recovered from the mucosa, it appeared that the oil was either being metabolized *in situ*, or that active transport was taking place. Accordingly lymph was collected from the intestinal lymphatics during the absorption of liquid paraffin, using the technique described by Bollman, Cain & Grindlay (1948). The unsaponifiable fraction yielded a wax, m.p. 25–26° (C, 65·9 and H, 10·4). Similar material was not obtained after feeding olive oil, and it would appear that the paraffin had been modified. This material was not cholesterol. The livers of animals kept for 15 months on a diet supplemented with 10 % (w/v) paraffin-olive oil contained 0·4 % dry-weight liquid paraffin (C, 86·5 and H, 13·1). It appears that active metabolism of the oil occurs. This has been suggested by Channon & Collinson (1929) and demonstrated by Stetten (1943). The continued ingestion of liquid paraffin has no deleterious effect on liver function as assessed by the method of Ahmad & Frazer (1952).

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Chemical Studies on the Gonadotrophin in Urine of Post-menopausal Women. By W. R. Butt and R. E. Deriaz.* (*Department of Clinical Endocrinology, Showell Green Lane, United Birmingham Hospitals*)

Gonadotrophin in the urine of women past the menopause is extracted by kaolin (Dekanski, 1949) and is purified by adsorption chromatography on tricalcium phosphate (Swingle & Tiselius, 1951). The properties of the biologically active eluate, which has been called gonadotrophin A (GA) (Crooke & Butt, 1952), have been studied by electrophoresis on filter-paper strips, by paper-partition chromatography of a neutralized acid hydrolysate, by polarographic detection of cystine and by colorimetric determinations of hexose and hexosamine. GA is a mucoprotein and differs from pituitary FSH in having an isoelectric point lower than 4·5. It resembles pituitary FSH in containing hexosamine, mannose, galactose and cystine. By comparison the urinary gonadotrophin from pregnant women has an isoelectric point of 3·2–3·3 (Li, 1949) and contains no cystine (Li, 1949).

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Studies of the Inositol-containing Lipids of Soya Bean. By E. Chargaff and J. N. Hawthorne. (*College of Physicians and Surgeons, New York* 32, *and Pharmacology Department, Medical School, Birmingham* 15)

The inositol-containing lipid present in soya bean (Woolley, 1943; Folch, 1947) has been prepared and purified by two methods. The first involved repeated precipitation from organic solvents, with methyl and ethyl alcohols, followed by suspension in N-HCl and dialysis. The second was similar, except that the lipid was first extracted with glacial acetic acid and partitioned between cyclohexane and dilute acetic acid, as suggested by Carter, Celmer, McCormick, Nyman & Saunders (1949). In the second preparation the lipid material soluble in acetic acid was also recovered and purified.

The preparations contained 3·6 % P after thorough dialysis, and 3·12 % before such treatment. The N:P ratio varied from 1:1·7 to 1:3·4 in different samples. Prolonged hydrolysis liberated

ethanolamine and about ten amino-acids. It may be that these substances arise from contaminating phosphatides and proteolipid material, rather than the inositol lipid itself.

Paper chromatography in 6:3:1 propanol-ammonia-water mixtures of the water-soluble phosphates produced by mild acid or alkaline hydrolysis of the preparations revealed glycerophosphate, inositol monophosphate and a slow-moving component, the structure of which is as yet unknown. This last may be a mixture of substances; it only occurred in traces in the acetic acid-soluble material, though inositol monophosphate was present in the usual amount. The unknown component was dextrorotatory (a fact noted by Klenk & Sakai (1939)) and non-reducing. On hydrolysis, however, inositol phosphate and at least three reducing sugars were produced. These latter included galactose and a pentose. The evidence suggests that the unknown component is a mixture of inositol phosphate glycosides. It is probable that the original 'inositol lipid' is a mixture itself.

We wish to thank Mr J. Eichberg of the American Lecithin Company for generous gifts of soya bean phosphatides.

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DEMONSTRATIONS

X-ray Diffraction Studies on Nerve Myelin. By J. B. Finean (introduced by A. C. Frazer)

Qualitative Chromatography of Long-chain Fatty-acids. By R. G. Baker (introduced by A. C. Frazer). (*Departments of Pharmacology and Experimental Psychiatry, University of Birmingham*)

A reversed phase partition system has been developed for the separation and identification of long-chain fatty-acids. Cellulose in the form of Whatman paper strips (3 mm.) is converted into the octadecyloxymethyl ether derivative, details of which are given in the patent literature. The paper is then water repellent, and is little changed in strength and texture. A ternary two-phase solvent system is used to develop the chromatogram. Dekalin forms the stationary phase and methanol/water the moving phase. The procedure used is as follows.

The paper is thoroughly dried by heating for 3 hr. in a thermostatically controlled oven, at 105°.

The strips are then immersed in toluene containing octadecyloxymethyl pyridinium chloride (0·5 %, w/v) and left at 90° for at least 3 hr.

After draining, the strips are washed (countercurrent) with five changes of absolute ethanol.

They are dried, and then boiled in distilled water to test for water repellency.

The paper strips can be used at this stage for chromatograms, but to avoid the formation of acidic fronts it is advisable to pre-wash with the developing solvent for at least 24 hr. (i.e. run as a blank chromatogram).

The stationary phase is applied either by spraying with dekalin, or by dipping the strip into a 1:1 dekalin/ether mixture. The solution of fatty acids is applied before spraying or after dipping. Approximately 30–50 μg. for each component should be used.

The chromatogram is developed at room temperature by the descending technique in a glass tank sealed with a plate-glass lid. Methanol/water mixtures, 80/20, 85/15, 90/10 v/v (equilibrated with dekalin), give good separations for the range C_{12}–C_{18}. The development time varies from 20 to 40 hr. and is proportional to the amount of water in the moving phase. The temperature of the tank should be maintained reasonably constant, particularly with 85/15 and 90/10 solvents.

The strips are dried in a warm oven and then sprayed with an alkaline 0·4 % solution of bromothymol blue in methyl cellosolve. The fatty-acid components show up as yellow spots on a blue background. The rate of movement is inversely related to chain length.

The quantitative application of the system is being investigated; the procedure followed in broad outline is that described by Howard & Martin (1950) for the dichlorodimethylsilane-treated kieselguhr columns.

REFERENCES

Preparation of chlormethyl ethers. B.P. 394, 196.
Use of octadecyloxymethylpyridinium chloride to confer water repellency. B.P. 469, 476.
Howard, G. A. & Martin, A. J. P. (1950). *Biochem. J.* **46**, 532.

FORTHCOMING PAPERS

It is hoped to publish the following papers in the next issue of the *Biochemical Journal*:

The application of countercurrent methods to the fractionation of lipid material from human placenta. By P. G. Cole, G. H. Lathe and C. R. J. Ruthven

The oxidative pathway of carbohydrate metabolism in *Escherichia coli*:

1. The isolation and properties of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. By Dwight B. McNair Scott and S. S. Cohen

2. Quantitative studies of glucose 6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase. By Dwight B. McNair Scott and S. S. Cohen

Observations on the fate of some aliphatic sulphonic acids in the rat. By G. A. Maw

The oxidation of dimethylthetin and related compounds to sulphate in the rat. By G. A. Maw

The estimation of D- and L-glutamic acid in proteins. By G. H. Wiltshire

The enzymic hydrolysis of alkyl fluoroacetates and related compounds. By F. Bergmann and Arela Shimoni

A dynamic osmometer for accurate measurements on small quantities of material: osmotic pressures of isoelectric β-lactoglobulin solutions. By O. Smithies

Studies on cholinesterase. 9. Species variation in the specificity pattern of the pseudo cholinesterases. By D. K. Myers

An apparatus for the spectrokinetic study of rapid reactions. By K. Dalziel

Appendix—The precision of analysis by photoelectric spectrophotometry. By K. Dalziel

Mould 'glucosaccharase': a fructosidase. By F. J. Bealing

Separation of uroporphyrin esters I and III by paper chromatography. By J. E. Falk and Amy Benson

Identification and classification of some porphyrins on the basis of their X-ray diffraction patterns. By Olga Kennard and C. Rimington

Studies on the 'Waldenström porphyrin' of acute porphyria urines. By R. E. H. Nicholas and C. Rimington

The protein components of the isolated myofibril. By S. V. Perry

A new colorimetric reagent for carbohydrates. By E. Lunt and D. Sutcliffe

Spectroscopic studies of carbohydrates. 1. The action of sulphuric acid on sugars. By R. M. Love

Studies on influenza virus receptor-substance and receptor-substance analogues. 1. Preparation and properties of a homogeneous mucoid from the salivary gland of sheep. By J. F. McCrea

Addendum—Physicochemical examination of a mucoid from the salivary gland of sheep. By E. A. Caspary

The proportion of phosphatase activity demonstrable in brain by histological techniques. By O. E. Pratt

Studies in detoxication:

51. The determination of catechols in urine, and the formation of catechols in rabbits receiving halogenobenzenes and other compounds. Dihydroxylation *in vivo*. By W. M. Azouz, D. V. Parke and R. T. Williams

52. The apparent dissociation constants of some glucuronides, mercapturic acids and related compounds. By D. Robinson, J. N. Smith and R. T. Williams

53. The glucuronic acid conjugation of hydroxyquinolines and hydroxypyridines in the rabbit. By J. N. Smith

The effect of pH on the affinities of enzymes for substrates and inhibitors. By M. Dixon

The determination of enzyme inhibitor constants. By M. Dixon

Studies on fumarase. 4. The effects of inhibitors on fumarase activity. By V. Massey

Absorption of immune globulin by the young lamb after ingestion of colostrum. By E. F. McCarthy and E. I. McDougall

The production of fatty acids by a gram-negative coccus. By S. R. Elsden and D. Lewis

The biosynthesis and metabolism of betaines in plants. 1. The estimation and distribution of glycinebetaine (betaine) in *Beta vulgaris* L. and other plants. By B. T. Cromwell and S. D. Rennie

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